



CHEMICAL PRODUCT DEVELOPMENT
AND DEFENSE

MR # 326202

L10-212

Contains Confidential Business Information

April 14, 2010

Via Facsimile

Ms. Virginia Lee
United States Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Mail Code: 7405M
Washington, D.C. 20460

COMPANY SANITIZED

Re: PMN Submission [REDACTED]

Dear Ms. Lee:

On behalf of our client, [REDACTED], The Acta Group, L.L.C. (Acta) confirms that [REDACTED] wishes to convert pending pre-manufacture notice [REDACTED] to a low volume exemption (LVE) application. With this letter, Acta understands that the documents submitted yesterday to the U.S. Environmental Protection Agency are sufficient to accomplish this change.

If you have any questions or comments, please call me at (202) 266-5031.

Sincerely,

A handwritten signature in cursive script that reads "Sheryl Lindros Dolan".

Sheryl Lindros Dolan

The Acta Group, L.L.C.
1203 Nineteenth Street, N.W.
Suite 300
Washington, D.C. 20036
TEL: 202/266-5020 • FAX: 202/557-3836
WEB: WWW.ACTAGROUP.COM

The Acta Group EU, Ltd
Avanta Royal Mills
17 Redhill Street
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Registered in England No. 5307852
Registered office: The Acta Group EU, Ltd. c/o PKF (UK).
LLP Sovereign House, Queen Street, Manchester M2 5HR



CHEMICAL PRODUCT DEVELOPMENT
AND DEFENSE

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April 13, 2010

Via Facsimile

Ms. Virginia Lee
United States Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Mail Code: 7405M
Washington, D.C. 20460

Re: PMN Submission [REDACTED]

Dear Ms. Lee:

On behalf of our client, we are forwarding the following materials as discussed:

- Confidential and sanitized replacement pages for EPA Form 7710-25; and
- Confidential and sanitized copies of a Material Safety Data Sheet revised to reflect EPA's comments.

If you have any questions or comments, I can be reached at (202) 266-5031.

Sincerely,

Sheryl Lindros Dolan



Attachments

The Acta Group, L.L.C.
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U.S. ENVIRONMENTAL PROTECTION AGENCY		Form Approved. O.M.B. No. 2070-0012. Approval Expires 10-31-96																															
 PREMANUFACTURE NOTICE  5 3 1 0 0 0 0 0 2 1 2 / S		AGENCY USE ONLY Date of receipt DEC 29 2009 COMPANY SANITIZED																															
Enter the total number of pages in the Premanufacture Notice 1,368		Document control number 53100000212 EPA case number L-10-212																															
GENERAL INSTRUCTIONS																																	
<ul style="list-style-type: none">You must provide all information requested in this form to the extent that it is known to or reasonably ascertainable by you. Make reasonable estimates if you do not have actual data.Before you complete this form, you should read the "Instructions Manual for Premanufacture Notification" (the Instructions Manual is available from the Toxic Substances Control Act (TSCA) Information Service by calling 202-554-1404, or faxing 202-554-5603).If a user fee has been remitted for this notice (40 CFR 700.45), indicate in the boxes above the TS-user fee identification number you have generated. Remember, your user fee ID number must also appear on your corresponding fee remittance, which is sent to EPA, Washington Financial Management Center (3303), P.O. 360399M, Pittsburgh, PA 15251-6399, Attn. TSCA User fee.																																	
Part I — GENERAL INFORMATION		TS - R 9 G F 0 2																															
<p>You must provide the currently correct Chemical Abstracts (CA) Name of the new chemical substance, even if you claim the identity as confidential. You may authorize another person to submit chemical identity information for you, but your submission will not be complete and the review will not begin until EPA receives this information. A letter in support of your submission should reference your TS user fee identification number. You must submit an original and two copies of this notice including all test data. If you claimed any information as confidential, a single sanitized copy must also be submitted.</p> <p>Part II — HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE</p> <p>If there are several manufacture, processing, or use operations to be described in Part II, sections A and B of this notice, reproduce the sections as needed.</p> <p>Part III — LIST OF ATTACHMENTS</p> <p>Attach additional sheets if there is not enough space to answer a question fully. Label each continuation sheet with the corresponding section heading. In Part III, list these attachments, any test data or other data and any optional information included in the notice.</p> <p>OPTIONAL INFORMATION</p> <p>You may include any information that you want EPA to consider in evaluating the new substance. On page 11 of this form, space has been provided for you to described pollution prevention and recycling information you may have regarding the new substance.</p> <p>So-called "binding" boxes are included throughout this form for you to indicate your willingness to be bound to certain statements you make in this section, such as use, production volume, protective equipment . . . This option is intended to reduce delays that routinely accompany the development of consent orders or Significant New Use Rules. Except in the case of exemption applications (such as TMEA, LVE, LOREX) where certain information provided in such notification is binding on the submitter when the Agency approves the exemption application, checking a binding box in this notice does not by itself prohibit the submitter from later deviating from the information (except chemical identity) reported in the form.</p> <p>CONFIDENTIALITY CLAIMS</p> <p>You may claim any information in this notice as confidential. To assert a claim on the form, mark (X) the confidential box next to the information that you claim as confidential. To assert a claim in an attachment, circle or bracket the information you claim as confidential. <u>If you claim information in the notices as confidential, you must also provide a sanitized version of the notice, (including attachments).</u> For additional instructions on claiming information as confidential, read the Instructions Manual.</p>		<p>TEST DATA AND OTHER DATA</p> <p>You are required to submit all test data in your possession or control and to provide a description of all other data known to or reasonably ascertainable by you, if these data are related to the health and environmental effects on the manufacture, processing, distribution in commerce, use, or disposal of the new chemical substance. Standard literature citations may be submitted for data in the open scientific literature. <u>Complete test data (written in English), not summaries of data, must be submitted if they do not appear in the open literature.</u> You should clearly identify whether test data is on the substance or on an analog. Also, the chemical composition of the tested material should be characterized. Following are examples of test data and other data. Data should be submitted according to the requirements of §720.50 of the Premanufacture Notification Rule (40 CFR Part 720).</p> <p>Test Data (Check Below any included in this notice)</p> <table border="0" style="width: 100%;"><tr><td>• Environmental fate data</td><td><input checked="" type="checkbox"/> Yes</td><td>Other data</td><td><input type="checkbox"/> Yes</td></tr><tr><td>• Health effects data</td><td><input checked="" type="checkbox"/> Yes</td><td>Risk assessments</td><td><input checked="" type="checkbox"/></td></tr><tr><td>• Environmental effects data</td><td><input checked="" type="checkbox"/> Yes</td><td>Structure/activity relationship</td><td><input checked="" type="checkbox"/></td></tr><tr><td>• Physical/Chemical Properties*</td><td><input checked="" type="checkbox"/> Yes</td><td>Test data not in the possession or control of the submitter</td><td><input type="checkbox"/></td></tr></table> <p>* A physical and chemical properties worksheet is located on the last page of this form.</p> <p>TYPE OF NOTICE (Check Only One)</p> <table border="0" style="width: 100%;"><tr><td><input type="checkbox"/> PMN (Premanufacture Notice)</td><td></td></tr><tr><td><input type="checkbox"/> INTERMEDIATE PMN (submitted in sequence with final product PMN)</td><td></td></tr><tr><td><input type="checkbox"/> SNUN (Significant New Use Notice)</td><td></td></tr><tr><td><input type="checkbox"/> TMEA (Test Marketing Exemption Application)</td><td></td></tr><tr><td><input checked="" type="checkbox"/> LVE (Low Volume Exemption) @ 40 CFR 723.50(c)(1)</td><td></td></tr><tr><td><input type="checkbox"/> LOREX (Low Release/Low Exposure Exemption) @ 40 CFR 723.50(c)(2)</td><td></td></tr><tr><td><input type="checkbox"/> LVE Modification</td><td><input type="checkbox"/> LOREX Modification</td></tr></table> <p>IS THIS A CONSOLIDATED PMN? <input type="checkbox"/> Yes</p> <p># of chemicals _____ (Prenotice Communication # required, enter # on page 3)</p>		• Environmental fate data	<input checked="" type="checkbox"/> Yes	Other data	<input type="checkbox"/> Yes	• Health effects data	<input checked="" type="checkbox"/> Yes	Risk assessments	<input checked="" type="checkbox"/>	• Environmental effects data	<input checked="" type="checkbox"/> Yes	Structure/activity relationship	<input checked="" type="checkbox"/>	• Physical/Chemical Properties*	<input checked="" type="checkbox"/> Yes	Test data not in the possession or control of the submitter	<input type="checkbox"/>	<input type="checkbox"/> PMN (Premanufacture Notice)		<input type="checkbox"/> INTERMEDIATE PMN (submitted in sequence with final product PMN)		<input type="checkbox"/> SNUN (Significant New Use Notice)		<input type="checkbox"/> TMEA (Test Marketing Exemption Application)		<input checked="" type="checkbox"/> LVE (Low Volume Exemption) @ 40 CFR 723.50(c)(1)		<input type="checkbox"/> LOREX (Low Release/Low Exposure Exemption) @ 40 CFR 723.50(c)(2)		<input type="checkbox"/> LVE Modification	<input type="checkbox"/> LOREX Modification
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Public reporting burden for this collection of information is estimated to average 110 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Director, Collection Strategies Division (2822), U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., N.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Act (2070-0012), Washington, D.C. 20503.

CERTIFICATION -- A Printed copy of this signature page, with original signature, must be submitted

I certify that to the best of my knowledge and belief:

1. The company named in Part I, section A, subsection 1a of this notice form intends to manufacture or import for a commercial purpose, other than in small quantities solely for research and development, the substance identified in Part I, Section B.
2. All information provided in this notice is complete and truthful as of the date of submission.
3. I am submitting with this notice all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by §720.50 of the Premanufacture Notification Rule.

Additional Certification Statements:

If you are submitting a PMN, Intermediate PMN, Consolidated PMN, or SNUN, check the following **user fee** certification statement that applies:

- ☐ The Company named in Part I, Section A has remitted the fee of \$2500 specified in 40 CFR 700.45(b), or
- ☐ The Company named in Part I, Section A has remitted the fee of \$1000 for an Intermediate PMN (defined @ 40 CFR 700.43) in accordance with 40 CFR 700.45(b), or
- ☐ The Company named in Part I Section A is a small business concern under 40 CFR 700.43 and has remitted a fee of \$100 in accordance with 40 CFR 700.45(b).

If you are submitting a **low volume exemption (LVE)** application in accordance with 40 CFR 723.50(c)(1) or a **Low release and low exposure exemption (LoRex)** application in accordance with 40 CFR 723.50(c)(2), check the following certification statements:

- ☒ The manufacturer submitting this notice intends to manufacture or import the new chemical substance for commercial purposes, other than in small quantities solely for research and development, under the terms of 40 CFR 723.50.
- ☒ The manufacturer is familiar with the terms of this section and will comply with those terms; and
- ☒ The new chemical substance for which the notice is submitted meets all applicable exemption conditions.
- ☒ If this application is for an LVE in accordance with 40 CFR 723.50(c)(1), the manufacturer intends to commence manufacture of the exempted substance for commercial purposes within 1 year of the date of the expiration of the 30 day review period.

The accuracy of the statements you make in this notice should reflect your best prediction of the anticipated facts regarding the chemical substance described herein. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 USC 1001.

Signature and title of Authorized Official (Original Signature Required)	Date	Confidential
		<input checked="" type="checkbox"/>
Signature of agent - (if applicable)	Date	<input type="checkbox"/>

Part I -- GENERAL INFORMATION

Section A -- SUBMITTER IDENTIFICATION				Confidential
Mark () the "Confidential" box next to any subsection you claim as confidential				
1a. Person Submitting Notice (in U.S.)	Name of authorized official		Position	<input checked="" type="checkbox"/>
	Company			
	Mailing address (number and street)			
	City, State	Postal Code		
b. Agent (if applicable)	Name of authorized official		Position	
	Company			
	Mailing address (number and street)			
	City, State area code)	Postal Code	Telephone (include	
c. If you are submitting this notice as part of a joint submission, mark (X) this box. <input type="checkbox"/>				
Joint Submitter (if applicable)	Name of authorized official		Position	
	Company			
	Mailing address (number and street)		City, State	
	Province, Country	Postal Code	Telephone (include area code)	
2. Technical Contact (in U.S.)	Name of authorized official		Position	
	Joseph E. Plamondon, Ph.D.		Senior Scientist	
	Company			
	Mailing address (number and street)			
		1203 Nineteenth Street, N.W., Suite 300		
City, State		Postal Code	Telephone (include area code)	
Washington, D.C.		20036-2401	(520) 572-3948	
3.	If you have had a prenotice communication (PC) concerning this notice and EPA assigned a PC Number to the notice, enter the number. <input type="text"/>		Mark (X) if none <input type="checkbox"/>	<input checked="" type="checkbox"/>
4.	If you previously submitted an exemption application for the chemical substance covered by this notice, enter the exemption number assigned by EPA. If you previously submitted a PMN for this substance enter the PMN number assigned by EPA (i.e. withdrawn or incomplete). <input type="text"/>		Mark (X) if none <input type="checkbox"/>	<input checked="" type="checkbox"/>
5.	If you have submitted a notice of Bona fide intent to manufacture or import for the chemical substance covered by this notice, enter the notice number assigned by EPA. <input type="text"/>		Mark (X) if none <input type="checkbox"/>	<input checked="" type="checkbox"/>
6.	Type of Notice - Mark (X)	1. <input type="checkbox"/> Manufacture Only <input type="checkbox"/> Binding Option Mark (X)	2. <input checked="" type="checkbox"/> Import Only <input type="checkbox"/> Binding Option Mark (X)	3. <input type="checkbox"/> Both

Part I – GENERAL INFORMATION – Continued			
Section B – CHEMICAL IDENTITY INFORMATION:		You must provide a currently correct Chemical Abstracts (CA) name of the substance based on the ninth Collective Index (9CI) of CA nomenclature rules and conventions.	
Mark (X) the "Confidential" box next to any item you claim as confidential			
<p>Complete either item 1 (Class 1 or 2 substances) or 2 (Polymers) as appropriate. Complete all other items.</p> <p>If another person will submit chemical identity information for you (for either Item 1 or 2), mark (X) the box at the right.</p> <p>Identify the name, company, and address of that person in a continuation sheet. → <input type="checkbox"/> </p>			
1. Class 1 or 2 chemical substances (for definitions of class 1 and class 2 substances, see the Instructions Manual)			Confidential
a. Class of substance - Mark (X)	<input type="checkbox"/> Class 1 or <input type="checkbox"/> Class 2		<input checked="" type="checkbox"/>
b. Chemical name (Currently correct Chemical Abstracts (CA) Name that is consistent with TSCA Inventory listings for similar substances. For Class 1 substances a CA Index Name must be provided. For Class 2 substances either a CA Index Name or CA Preferred Name must be provided, which ever is appropriate based on CA 9CI nomenclature rules and conventions).			<input checked="" type="checkbox"/>
c. Please identify which method you used to develop or obtain the specified chemical identity information reported in this notice: (check one).			
<input type="checkbox"/> Method 1 (CAS Inventory Expert Service - a copy of the Identification report obtained from the CAS Inventory Expert Services must be submitted as an attachment to this notice)	<input type="checkbox"/> Method 2 (Other Source)		<input checked="" type="checkbox"/>
d. Molecular formula	CBI <input checked="" type="checkbox"/>	CAS Registry Number (if a number already exists for the substance)	<input checked="" type="checkbox"/>
For a class 1 substance, provide a complete and correct chemical structure diagram. For a class 2 substance, provide a correct representative or partial chemical structure diagram, as complete as can be known, if one can be reasonably ascertained. Please see the E-PMN Instruction Manual for discussion of "native format" diagram software which can be helpful in reviewing your substance.			<input checked="" type="checkbox"/>
<input type="checkbox"/> Mark (X) this box if you attach a continuation sheet.			

Part I -- GENERAL INFORMATION -- Continued

Section B -- CHEMICAL IDENTITY INFORMATION -- Continued

2. Polymers (For a definition of polymer, see the Instructions Manual.)

Confidential

- a. Indicate the number-average weight of the lowest molecular weight composition of the polymer you intend to manufacture. Indicate maximum weight percent of low molecular weight species (not including residual monomers, reactants, or solvents) below 500 and below 1,000 absolute molecular weight of that composition.

Describe the methods of measurement or the basis for your estimates: GPC ☐ Other ☐: (Specify below)

- i) lowest number average molecular weight: _____
 ii) maximum weight % below 500 molecular weight: _____
 iii) maximum weight % below 1000 molecular weight: _____

☐ Mark (X) this box if you attach a continuation sheet.

- b. You must make separate confidentiality claims for monomer or other reactant identity, composition information, and residual information. Mark (X) the "Confidential" box next to any item you claim as confidential
- (1) - Provide the specific chemical name and CAS Registry Number (if a number exists) of each monomer or other reactant used in the manufacture of the polymer.
 - (2) - Mark (X) this column if entry in column (1) is confidential.
 - (3) - Indicate the typical weight percent of each monomer or other reactant in the polymer.
 - (4) - Type "yes" in the identity column if you want a monomer or other reactant used at two weight percent or less to be listed as part of the polymer description on the TSCA Chemical Substance Inventory.
 - (5) - Mark (X) this column if entries in columns (3) and (4) are confidential.
 - (6) - Indicate the maximum weight percent of each monomer or other reactant that may be present as a residual in the polymer as manufactured for commercial purposes.
 - (7) - Mark (X) this column if entry in column (6) is confidential.

Monomer or other reactant and CAS Registry Number (1)	Confidential (2)	Typical composition (3)	Include in Identity (4)	Confidential (5)	Maximum residual (6)	Confidential (7)
		%			%	
		%			%	
		%			%	
		%			%	
		%			%	
		%			%	
		%			%	

☐ Mark (X) this box if you attach a continuation sheet.

- c. Please identify which method you used to develop or obtain the specified chemical identity information reported in this notice (check one).

☐ Method 1 (CAS Inventory Expert Service - a copy of the identification report source) ☐ Method 2 (other source)
 obtained from CAS Inventory Expert Service must be submitted as an attachment to this notice)

CBI

- d. The currently correct Chemical Abstracts (CA) name for the polymer that is consistent with TSCA Inventory listings for similar polymers.

- e. Provide a correct representative or partial chemical structure diagram, as complete as can be known, if one can be reasonably ascertained. Please see the E-PMN Instruction Manual for discussion of "native format" diagram software which can be helpful in reviewing your substance.

☐ Mark (X) this box if you attach a continuation sheet.

Part I -- GENERAL INFORMATION -- Continued**Section B -- CHEMICAL IDENTITY INFORMATION -- Continued****3. Impurities**

- (a) - Identify each impurity that may be reasonably anticipated to be present in the chemical substance as manufactured for commercial purpose. Provide the CAS Registry Number if available. If there are unidentified impurities, enter "unidentified."
- (b) - Estimate the maximum weight % of each impurity. If there are unidentified impurities, estimate their total weight %.

Impurity and CAS Registry Number (a)	Maximum percent (b)	Confidential
	%	
	%	
	%	
	%	
	%	
	%	
	%	

☐ Mark (X) this box if you attach a continuation sheet.
4. Synonyms - Enter any chemical synonyms for the new chemical identified in subsection 1 or 2.

Confidential

☐ Mark (X) this box if you attach a continuation sheet.
5. Trade identification - List trade names for the new chemical substance identified in subsection 1 or 2.
☐ Mark (X) this box if you attach a continuation sheet.
6. Generic chemical name - If you claim chemical identity as confidential, you must provide a generic name for your substance that reveals the specific chemical identity of the new chemical substance to the maximum extent possible. Refer to the TSCA Chemical Substance Inventory, 1985 Edition, Appendix B for guidance on developing generic names.**Polycyclic polyamine diester organometallic compound**
☐ Mark (X) this box if you attach a continuation sheet.
7. Byproducts - Describe any byproducts resulting from the manufacture, processing, use, or disposal of the new chemical substance. Provide the CAS Registry Number if available.

Byproduct (1)	CAS Registry Number (2)	Confidential

☐ Mark (X) this box if you attach a continuation sheet.

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Part I -- GENERAL INFORMATION -- Continued												
Section C -- PRODUCTION, IMPORT, AND USE INFORMATION:												
Mark (X) the "Confidential" box next to any item you claim as confidential.												
1. Production volume -- Estimate the maximum production volume during the first 12 months of production. Also estimate the maximum production volume for any consecutive 12-month period during the first three years of production. Estimates should be on 100% new chemical substance basis. For a Low Volume Exemption application, if you choose to have your notice reviewed at a lower production volume than 10,000 kg/yr, specify the volume and mark (x) in the binding box. If granted, you are bound to this volume												
Maximum first 12-month production (kg/yr) (100% new chemical substance basis)						Maximum 12-month production (kg/yr) (100% new chemical substance basis)			Confidential	Binding Option Mark (x)		
									<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
2. Use Information -- You must make separate confidentiality claims for the description of the category of use, the percent of production volume devoted to each category, the formulation of the new substance, and other use information. Mark (X) the "Confidential" Box next to any item you claim as confidential.												
a. (1) -- Describe each intended category of use of the new chemical substance by function and application. (2) -- Mark (X) this column if entry column (1) is confidential business information (CBI). (3) -- Indicate your willingness to have the information provided in column (1) binding. (4) -- Estimate the percent of total production for the first three years devoted to each category of use. (5) -- Mark (X) this column if entry in column (4) is confidential business information (CBI). (6) -- Estimate the percent of the new substance as formulated in mixtures, suspensions, emulsions, solutions, or gels as manufactured for commercial purposes at sites under your control associated with each category of use. (7) -- Mark (X) this column if entry in column (6) is confidential business information (CBI). (8) -- Indicate % of product volume expected for the listed "use" sectors. Mark more than one box if appropriate. Mark (X) to indicate your willingness to have the use type provided in (8) binding. (9) -- Mark (X) this column if entry(ies) in column (8) is (are) confidential business information (CBI).												
Category of use (1) (by function and application i.e., a dispersive dye for finishing polyester fibers)	CBI (2)	Binding Option Mark (x) (3)	Produc- tion % (4)	CBI (5)	% in Form- ulation (6)	CBI (7)	% of substance expected per use (8)					CBI (9)
	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	Site- limited	Con- sumer	Industrial	Com- mercial	Binding Option	<input checked="" type="checkbox"/>
	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>						<input checked="" type="checkbox"/>
			%		%							
			%		%							
			%		%							
			%		%							
			%		%							
* If you have identified a "consumer" use, please provide on a continuation sheet a detailed description of the use(s) of this chemical substance in consumer products. In addition include estimates of the concentration of the new chemical substance as expected in consumer products and describe the chemical reactions by which this substance loses its identity in the consumer product.												
<input type="checkbox"/> Mark (X) this box if you attach a continuation sheet.												
b. Generic use descriptions If you claim any category of use description in subsection 2a as confidential, enter a generic description of that category. Read the Instructions Manual for examples of generic use descriptions.												
Coatings additive at a 1% concentration or less.												
<input type="checkbox"/> Mark (X) this box if you attach a continuation sheet.												
3. Hazard Information -- Include in the notice a copy of reasonable facsimile of any hazard warning statement, label, material safety data sheet, or other information which will be provided to any person who is reasonably likely to be exposed to this substance regarding protective equipment or practices for the safe handling, transport, use, or disposal of the new substance. List in part III hazard information you include.											Binding Option Mark (x)	
<input checked="" type="checkbox"/> Mark (X) this box if you attach hazard information.												

Part II—HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE**Section A – INDUSTRIAL SITES CONTROLLED BY THE SUBMITTER**

Mark (X) the "Confidential" box next to any item you claim as confidential

Complete section A for each type of manufacture, processing, or use operation involving the new chemical substance at industrial sites you control. Importers do not have to complete this section for operations outside the U.S.; however, you may still have reporting requirements if there are further industrial processing or use operations after import. You must describe these operations. See instructions manual

1. Operation description

a. Identity -- Enter the identity of the site at which the operation will occur.


Confidential

Name



Site address (number and street)

City, County, State, ZIP code

If the same operation will occur at more than one site, enter the number of sites. Identify the additional sites on a continuation sheet, and if any of the sites have significantly different production rates or operations, include all the information requested in this section for those sites as attachments. 

☐ Mark (X) this box if you attach a continuation sheet.**b. Type --**

Mark (X)



Manufacturing



Processing



Use

**c. Amount and Duration -- Complete 1 or 2 as appropriate**

1. Batch	Maximum kg/batch (100% new chemical substance)	Hours/batch	Batches/year	<input type="checkbox"/>
2. Continuous	Maximum kg/batch (100% new chemical substance)	Hours/day	Days/year	<input type="checkbox"/>

d. Process description

Mark (X) to indicate your willingness to have your process description binding.

- (1) Diagram the major unit operation steps and chemical conversions. Include interim storage and transport containers (specify- e.g. 5 gallon pails, 55 gallon drum, rail car, tank truck, etc.).
- (2) Provide the identity, the approximate weight (by kg/day or kg/batch on a 100% new chemical substance basis), and entry point of all starting materials and feedstocks (including reactants, solvents, catalysts, etc.), and of all products, recycle streams, and wastes. Include cleaning chemicals (note frequency if not used daily or per batch.).
- (3) Identify by number the points of release, including small or intermittent releases, to the environment of the new chemical substance.



Mark (X) this box if you attach a continuation sheet.

Part II—HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE – Continued**Section A -- INDUSTRIAL SITES CONTROLLED BY THE SUBMITTER – Continued**

2. Occupational Exposure -- You must make separate confidentiality claims for the description of worker activity, physical form of the new chemical substance, number of works exposed, and duration of activity. Mark (X) the "Confidential" box next to any item you claim as confidential.

- (1) -- Describe the activities (i.e. bag dumping, tote filling, unloading drums, sampling, cleaning, etc.) in which workers may be exposed to the substance.
 (2) -- Mark (X) this column if entry in column (1) is confidential business information (CBI).
 (3) -- Describe any protective equipment and engineering controls used to protect workers.
 (4) and (6) -- Indicate your willingness to have the information provided in column (3) or (5) binding.
 (5) -- Indicate the physical form(s) of the new chemical substance (e.g., solid, crystal, granule, powder, or dust) and % new chemical substance (if part of a mixture) at the time of exposure.
 (7) -- Mark (X) this column if entry in column (5) is confidential business information (CBI).
 (8) -- Estimate the maximum number of workers involved in each activity for all sites combined.
 (9) -- Mark (X) this column if entry in column (8) is confidential business information (CBI).
 (10) and (11) -- Estimate the maximum duration of the activity for any worker in hours per day and days per year.
 (12) -- Mark (X) this column if entries in columns (10) and (11) are confidential business information (CBI).

Worker activity (e.g., bag dumping, filling drums) (1)	CBI (2)	Protective Equipment/ Engineering Controls (3)	Binding Option Mark (x) (4)	Physical forms(s) (e.g., solid:powder) and % new substance (5)	Binding Option Mark (x) (6)	CBI (7)	# of Workers Exposed (8)	CBI (9)	Maximum Hrs/day (10)	duration Days/yr (11)	CBI (12)
Not applicable											

☐ Mark (X) this box if you attach a continuation sheet.

3. Environmental Release and Disposal -- You must make separate confidentiality claims for the release number and the amount of the new chemical substance released and other release and disposal information. Mark (X) the "Confidential" box next to each item you claim as confidential.

- (1) -- Enter the number of each release point identified in the process description, part II, section A, subsection 1d(3).
 (2) -- Estimate the amount of the new substance released (a) directly to the environment or (b) into control technology (in kg/day or kg/batch).
 (3) -- Mark (X) this column if entries in columns (1) and (2) are confidential business information (CBI).
 (4) -- Identify the media (stack air, fugitive air (optional-see Instruction Manual), surface water, on-site or off-site land or incineration, POTW, or other (specify)) to which the new substance will be released from that release point.
 (5) -- a. Describe control technology, if any, and control efficiency that will be used to limit the release of the new substance to the environment. For releases disposed of on land, characterize the disposal method and state whether it is approved for disposal of RCRA hazardous waste. On a continuation sheet, for each site describe any additional disposal methods that will be used and whether the waste is subject to secondary or tertiary on-site treatment. b. Estimate the amount released to the environment after control technology (in kg/day).
 (6) -- Mark (X) this column if entries in columns (4) and (5) are confidential business information (CBI).
 (7) -- Identify the destination(s) of releases to water. Please supply NPDES (National Pollutant Discharge Elimination System) numbers for direct discharges or NPDES numbers of the POTW (Publicly Owned Treatment Works). Mark (X) if the POTW name or NPDES # is confidential business information (CBI).

Release Number (1)	Amount of new substance released		CBI (3)	Media of release (e.g. stack air) (4)	Control technology and efficiency (you may wish to optionally attach efficiency data)			CBI (6)
	(2a)	(2b)			(5a)	Binding Mark (X)	(5b)	

(7) Mark (X) the destination(s) of releases to water	<input type="checkbox"/> below:	POTW provide name(s)	CBI <input type="checkbox"/>	<input type="checkbox"/> Navigable waterway	<input type="checkbox"/> Other - Specify	provide NPDES #	CBI <input type="checkbox"/>

☐ Mark (X) this box if you attach a continuation sheet.

Part II—HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE – Continued

Section B -- INDUSTRIAL SITES CONTROLLED BY OTHERS

Complete section B for typical processing or use operations involving the new chemical substance at sites you do not control. Importers do not have to complete this section for operations outside the U.S.; however, you must report any processing or use activities after import. See the Instructions Manual. *Complete a separate section B for each type of processing, or use operation involving the new chemical substance.* If the same operation is performed at more than one site describe the typical operation common to these sites. Identify additional sites on a continuation sheet.

- 1. Operation Description --** To claim information in this section as confidential, circle or bracket the specific information that you claim as confidential.
- (1) -- Diagram the major unit operation steps and chemical conversions, including interim storage and transport containers (specify - e.g. 5 gallon pails, 55 gallon drums, rail cars, tank trucks, etc). On the diagram, identify by letter and briefly describe each worker activity. (2) -- Either in the diagram or in the text field 1(b) below, provide the identity, the approximate weight (by kg/day or kg/batch, on an 100% new chemical substance basis), and entry point of all feedstocks (including reactants, solvents and catalysts, etc) and all products, recycle streams, and wastes. Include cleaning chemicals (note frequency if not used daily or per batch). (3) -- Either in the diagram or in the text field 1(b) below, identify by number the points of release, including small or intermittent releases, to the environment of the new chemical substance. (4) Please enter the # of sites (remember to identify the locations of these sites on a continuation sheet):

of sites

CBI



☐ Mark (X) this box if you attach a continuation sheet.

2. Worker Exposure/Environmental Release

- (1) -- From the diagram above, provide the letter for each worker activity. Complete 2-8 for each worker activity described.
- (2) -- Estimate the number of workers exposed for all sites combined.
- (4) -- Estimate the typical duration of exposure per worker in (a) hours per day and (b) days per year.
- (6) -- Describe physical form of exposure and % new chemical substance (if in mixture), and any protective equipment and engineering controls, if any, used to protect workers.
- (7) -- Estimate the percent of the new substance as formulated when packaged or used as a final product.
- (9) -- From the process diagram above, enter the number of each release point. Complete 9-13 for each release point identified.
- (10) -- Estimate the amount of the new substance released (a) directly to the environment or (b) into control technology to the environment (in kg/day or kg/batch).
- (12) -- Describe media of release i.e. stack air, fugitive air (optional-see Instructions Manual), surface water, on-site or off-site land or incineration, POTW, or other (specify) and control technology, if any, that will be used to limit the release of the new substance to the environment.
- (14) -- Identify byproducts which may result from the operation.
- (3), (5), (8), (11), (13) and (15) – Mark (X) this column if any of the proceeding entries are confidential business information (CBI).

Letter of Activity (1)	# of Workers Exposed (2)	CBI (3)	Duration of Exposure		CBI (5)	Protective Equip./ Engineering Controls/ Physical Form and % new substance (6)	% in Formulation (7)	CBI (8)	Release Number (9)	Amount of New Substances Released		CBI (11)	Media of Release & Control Technology (12)	CBI (13)
			(4a)	(4b)						(10a)	(10b)			

(14) - Byproducts: (15)

☐ Mark (X) this box if you attach a continuation sheet

[illegible]

Mark (X) this box if you attach a continuation sheet.

Attach continuation sheets for sections of the form and test data and other data (including physical/chemical properties and structure/activity information), and optional information after this page. Clearly identify the attachment and the section of the form to which it relates, if appropriate. Number consecutively the pages of the attachments. In the column below, enter the inclusive page numbers of each attachment. Electronic attachments can be identified by filename. Mark (X) the "Confidential" box next to any attachment name you claim as confidential. Read the Instructions Manual for guidance on how to claim any information in an attachment as confidential. You must include with the sanitized copy of the notice form a sanitized version of any attachment in which you claim information as confidential.

PHYSICAL AND CHEMICAL PROPERTIES WORKSHEET

To assist EPA's review of physical and chemical properties data, please complete the following worksheet for data you provide and include it in the notice. Identify the property measured, the page of the notice on which the property appears, the value of the property, the units in which the property is measured (as necessary), and whether or not the property is claimed as confidential. The physical state of the neat substance should be provided. These measured properties should be for the neat (100% pure) chemical substance. Properties that are measured for mixtures or formulations should be so noted (% PMN substance in ____). You are not required to submit this worksheet; however, EPA strongly recommends that you do so, as it will simplify review and ensure that confidential information is properly protected. You should submit this worksheet as a supplement to your submission of test data. This worksheet is not a substitute for submission of test data.

Property (a)	Mark (X) if provided	Page number (b)	Value (c)	Measured or Estimate (M or E)	Confidential Mark (X) (d)
Physical state of neat substance	X		____ (s) ____ (l) ____ (g)		<input checked="" type="checkbox"/>
Vapor pressure @ Temperature _____	X				<input checked="" type="checkbox"/>
Density/relative density	X				<input checked="" type="checkbox"/>
Solubility @ Temperature _____ °C Solvent _____			g/L		<input type="checkbox"/>
Solubility in water @ Temperature _____	X				<input checked="" type="checkbox"/>
Melting temperature					<input type="checkbox"/>
Boiling / sublimation temperature at ____ torr pressure					<input type="checkbox"/>
Spectra					<input type="checkbox"/>
Dissociation constant					<input type="checkbox"/>
Particle size distribution					<input type="checkbox"/>
Octanol / water partition coefficient	X				<input checked="" type="checkbox"/>
Henry's Law constant					<input type="checkbox"/>
Volatilization from water					<input type="checkbox"/>
Volatilization from soil					<input type="checkbox"/>
pH @ concentration _____					<input type="checkbox"/>
Flammability	X				<input checked="" type="checkbox"/>
Explosability	X				<input checked="" type="checkbox"/>
Adsorption / coefficient					<input type="checkbox"/>
Other -	X				<input checked="" type="checkbox"/>
Other -	X				<input checked="" type="checkbox"/>
Other -	X				<input checked="" type="checkbox"/>



Safety Data Sheet - US

Rev: [REDACTED]
Date: 12 April 2010

Date of printing: 13 April 2010

1. Identification of the substance/preparation and of the company/undertaking

Trade name

[REDACTED]

Use of the substance/preparation

Industry sector:

Type of use:

[REDACTED]
[REDACTED]

Identification of the company

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Information about the substance/preparation

[REDACTED]
[REDACTED]
[REDACTED]

2. Hazard identification

May cause sensitization by skin contact

3. Composition/information on ingredients

Chemical characterization

[REDACTED]

Hazardous ingredients

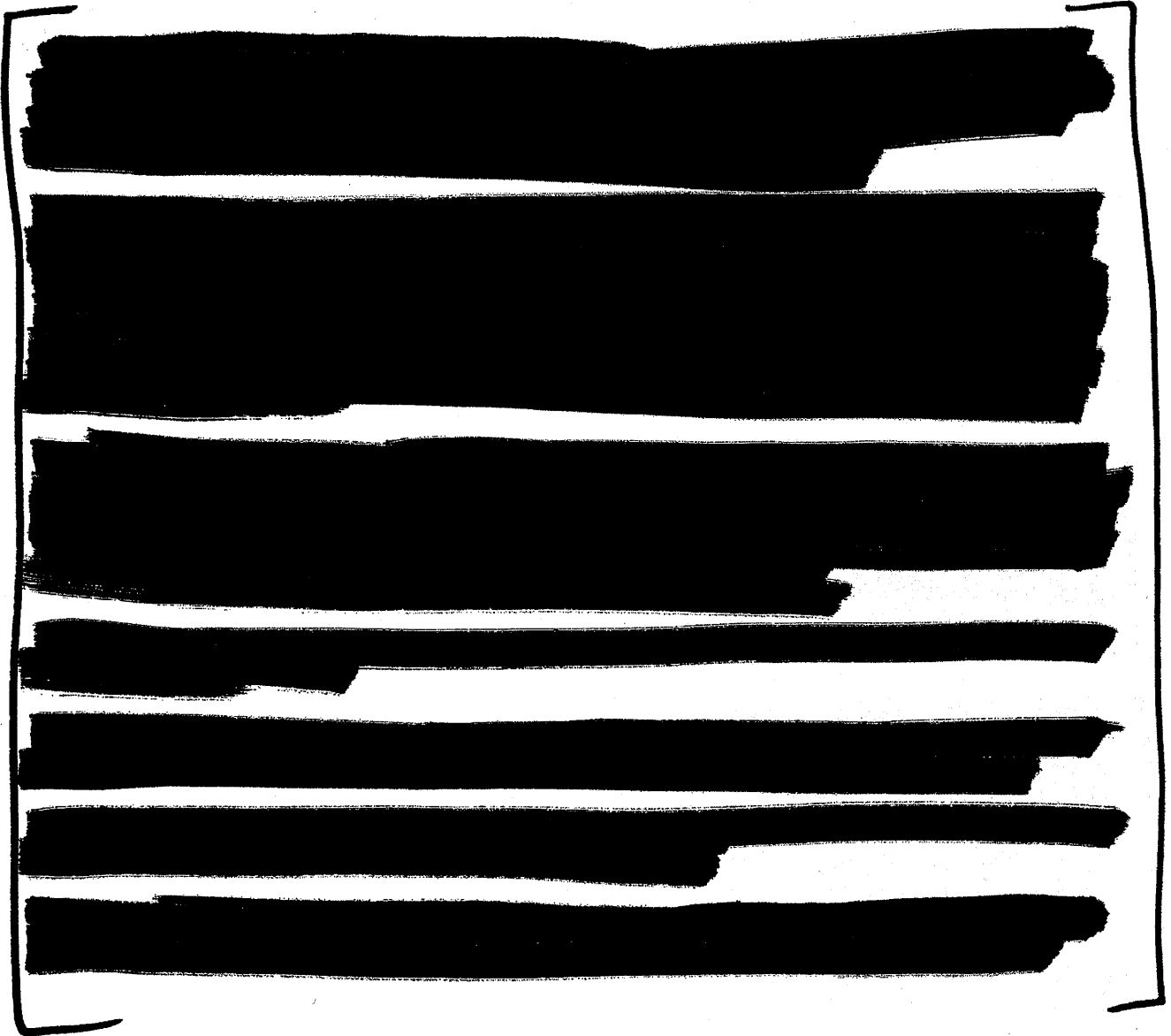
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

4. First aid measures

General information

Remove soiled or soaked clothing immediately

Sustainable Futures P2 Assessment for [REDACTED]



Sustainable Futures Summary Assessment Using P2 Framework Models

This document was developed to help compile estimation results from U.S. EPA OPPT's P2 Framework Models www.epa.gov/oppt/p2framework/ and is used by OPPT during Sustainable Futures (SF) training described at www.epa.gov/opptintr/newchemicals/sustainablefutures.htm. Participants in the voluntary SF Pilot Project are asked to submit the information contained in this assessment along with their SF PMNs in their choice of format.
Use of this specific format is not mandatory.

Chemical Assessed:

[REDACTED]

CAS Registry Number:

[REDACTED]

Participant Name:

[REDACTED]

Date of Assessment:

11/17/2009

[illegible]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1. The first part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

2. The second part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

3. The third part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

4. The fourth part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

5. The fifth part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

6. The sixth part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

7. The seventh part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

8. The eighth part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mrs. A. B. Jones", and "Mr. C. D. Brown".

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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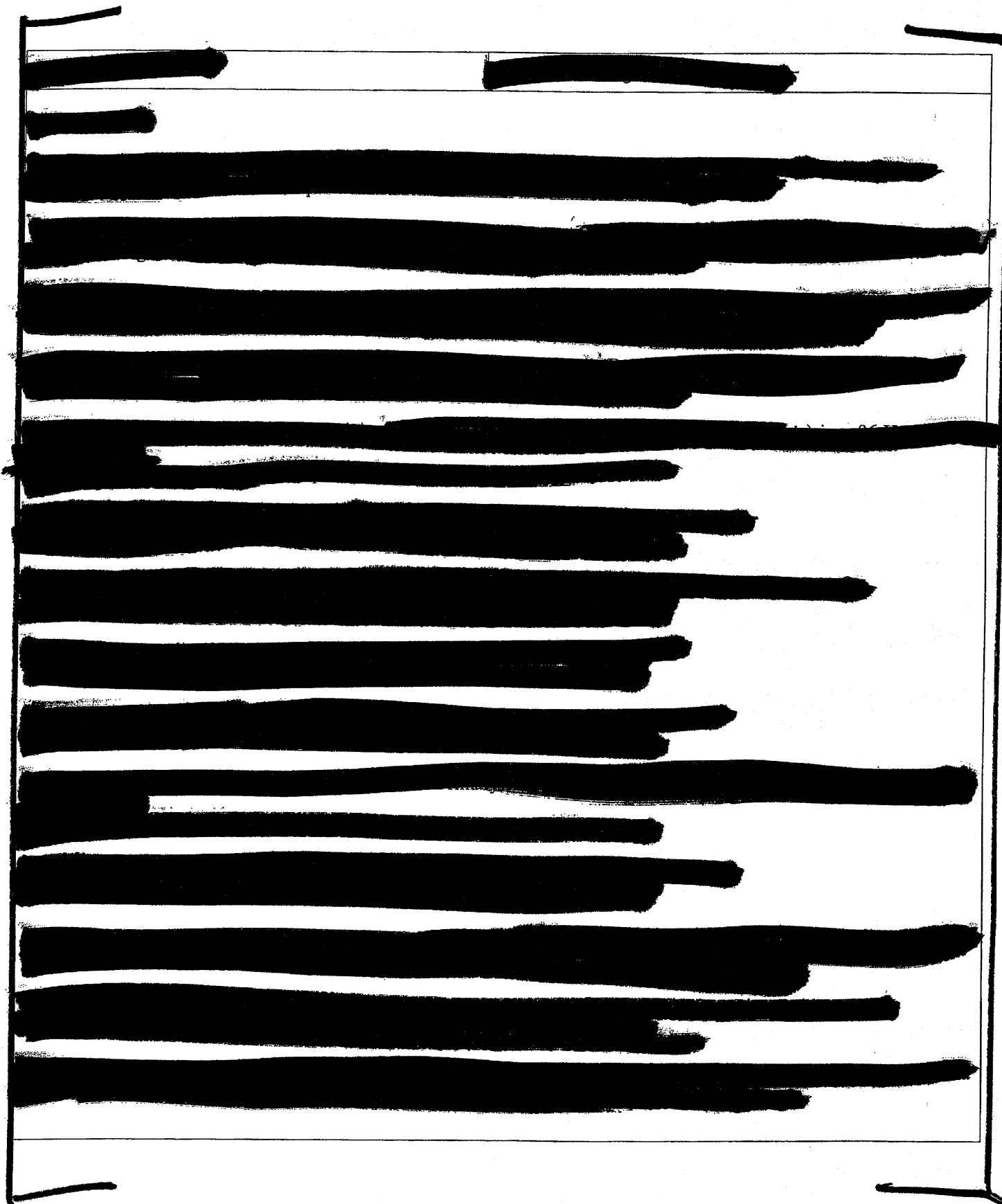
10. The tenth part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mr. W. H. Jones", and "Mr. A. B. Brown".

[REDACTED]

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STUDY REPORT

[REDACTED]
CHEMICAL CHARACTERISATION,
HOMOGENEITY AND STABILITY

Study AC030449

AUTHOR : CHRIS SPARHAM (STUDY DIRECTOR)

[REDACTED]
This report must not be circulated further, copied, or destroyed without
reference to the Reports Administrator [REDACTED]

[REDACTED]
Date : September 2008

STUDY INFORMATION

Study title : [REDACTED] Chemical characterisation, homogeneity
and stability

Study number : AC030449

Study location :

[REDACTED]
[REDACTED]

Butterworth Laboratories Ltd,
54-56, Waldergrave Road,
Teddington,
Middlesex,
TW11 8LG.

STUDY DATES

Date protocol signed : 28th October 2003

Experimental period : 29th October 2003 to 12th November 2004

ARCHIVING

Data & Report : Datacare Business Management Systems
3012 Heyford Park
Upper Heyford
Bucks
OX25 5HF

Test item(s) :

[REDACTED]

STUDY PERSONNEL

Study Director : Chris Sparham

STUDY INFORMATION (CONTINUED)

Responsible Practitioner : E. Findlay (Measurement Science)
Phase : [REDACTED]
Principal Investigator : D. Bell, (Butterworth Laboratories Ltd)
Phase : [REDACTED]
Analysts : Sean O'Connor, Nicola Bettles, Mark Tinkler,
Ian Bromilow

MONITORING ROLES

Scientific Reviewer : Neil Colson
Quality Assurance : Harjit Lall

CROSS REFERENCES

Project number : 221130

AUTHORISATION STATEMENT

Study number : AC030449
Study title : [REDACTED] Chemical characterisation, homogeneity
and stability

This report has been authorised for issue to the appropriate recipients.


NEIL COLSON
DEPARTMENT HEAD [REDACTED]

10/01/05
DATE

[REDACTED]

QUALITY ASSURANCE STATEMENT

Study Number : AC030449

Page 1 of 2

Study Title: [REDACTED] Chemical Characterisation, Homogeneity and Stability.

This study was conducted at [REDACTED] and Butterworth Laboratories Ltd, Teddington, Middlesex.

The following inspections and audits were conducted on the study. The dates on which they were performed and the dates on which any findings were reported to the Study Director and to Management are given below.

Audit Type	Audit Date	Report Date
Protocol Audit	28-Oct-2003	28-Oct-2003
Study Report Audit	13-Dec-2004	14-Dec-2004
Process Inspection	01-Sep-2003	27-Nov-2003
- Measurement Procedures – Wt/Vol.		
- HPLC		
- LCMS		
- UV/Vis		
- Total Volatiles		
Process Inspection	05-Jan-2004	19-Mar-2004
- Measurement Procedures – Wt/Vol.		
- LCMS		
- GCMS		
- HPLC		
Process Inspection	02-Feb-2004	18-Mar-2004
- Measurement Procedures - Wt/Vol		
- GCMS		
Procedural Inspection	14-Nov-2003	27-Nov-2003
- [REDACTED]		
Procedural Inspection	22-Jan-2004	30-Jan-2004
- [REDACTED]		
Procedural Inspection	26-Feb-2004	26-Feb-2004
- [REDACTED]		
<u>Facility Inspections</u>		
[REDACTED] Chemistry Department	27-Nov-2003	05-Dec-2003
[REDACTED] Product Support	19-Feb-2004	25-Feb-2004

[REDACTED]

07-May-2003

15-May-2003

Butterworth Laboratories Ltd.
Analytical Process Inspection

18-Nov-2003

18-Nov-2003

Analytical Results
- Elemental Analysis

18-Nov-2003

18-Nov-2003

Final Report

18-Nov-2003

18-Nov-2003

Page 2 of 2

As far as can reasonably be established, this report has been accepted by Quality Assurance as being an accurate presentation of the raw data and findings of the study.


H LALL

24 Dec 2004

DATE

QUALITY ASSURANCE


AUTHENTICATION STATEMENT

Study number : AC030449
Study title : [REDACTED] Chemical characterisation; homogeneity
and stability

I, the undersigned, hereby declare that this study has been conducted under my supervision, as Study Director, in accordance with [REDACTED] policy on Good Laboratory Practice which is based on the UK Good Laboratory Practice Regulations 1999, Statutory Instrument No. 3106 (amended by the Good Laboratory Practice (Codification Amendments etc) Regulations 2004, Statutory Instrument No. 994) and OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM/ (98)17.

I also certify that this report presents a true and accurate account of the procedures used and the results obtained.

Chris Sparham
CHRIS SPARHAM
STUDY DIRECTOR

7/1/05
DATE


[REDACTED]

RESPONSIBLE PRACTITIONER

Study number : AC030449

Study title : [REDACTED] Chemical characterisation, homogeneity
and stability

[REDACTED]









LIZ FINDLAY

7-1-05
DATE

[REDACTED]


CHEMICAL CHARACTERISATION,
HOMOGENEITY AND STABILITY

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[REDACTED]

CHEMICAL CHARACTERISATION,
HOMOGENEITY AND STABILITY

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[REDACTED]

CHEMICAL CHARACTERISATION,
HOMOGENEITY AND STABILITY

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[REDACTED]

CHEMICAL CHARACTERISATION,
HOMOGENEITY AND STABILITY

SUMMARY

[REDACTED] A characterisation study was required for the final representative material [REDACTED] produced by [REDACTED] in order to support the safety testing programme of this material. The intention of this study was to provide characterisation, homogeneity and stability data on [REDACTED]

[REDACTED] (S2539801) was characterised by a variety of techniques including liquid chromatography/mass spectrometry (LC/MS), liquid chromatography/ultraviolet detection (LC/UV), total volatiles, inductively coupled plasma/ atomic emission spectroscopy (ICP/AES) and [REDACTED]. The key results are summarised as follows:

From the [REDACTED] the experimentally derived formula was [REDACTED]. This was consistent with the proposed structure with the addition of 1 water molecule. The total volatile material, determined at 3.07 % w/w, accounted for the presence of this water (theoretically 2.7 % w/w). The purity of the main active (catalyst) by LC/UV at 260 nm was determined to be 97.89 %, on a peak area basis. The homogeneity of the bulk sample, by replicate analysis of 3 sub-samples, was also demonstrated by this technique. Two main impurities were seen in the LC/UV data, which were the [REDACTED] and the [REDACTED] of the main active (peak area 1.21 %). Structures of the main components of the sample were confirmed by LC/MS molecular ions. Other smaller impurities were tentatively identified by LC/MS. The amount of [REDACTED] present, determined by spiked addition LC/MS, was 1 % w/w which gave good agreement with the % area data. The sample was demonstrated to be stable over the course of the study, as stored at ambient temperature in the dark, by reanalysis using LC/MS and LC/UV.

Other comparisons carried out in the study showed BL 1749 Batch 005 (S2430001) to be very similar to S2539801 but with elevated levels of [REDACTED] (3.2 %) and no [REDACTED] (< 0.1%) as analysed by LC/UV on an area basis. The [REDACTED] (S2601501) was analysed to provide confirmation that there was no [REDACTED] present in the sample (< 0.04 % by LC/UV area).

1. INTRODUCTION

[REDACTED] A characterisation study was required for the final representative material [REDACTED] produced by [REDACTED] in order to support the safety testing programme of this material. The intention of this study was to provide characterisation, homogeneity and stability data on [REDACTED]

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2. SUBSTANCES TESTED**2.1 Test item**

Name	:	[REDACTED]
Sample Number	:	S2539801
Appearance	:	[REDACTED]
Storage	:	ambient/dark

Name	:	BL 1749 batch 005
Sample Number	:	S2430001
Appearance	:	[REDACTED]
Storage	:	ambient

Name	:	[REDACTED]
Sample Number	:	S2601501
Appearance	:	[REDACTED]
Storage	:	ambient/dark

Characterisation, stability and homogeneity of S2539801 is confirmed by analysis in this study. S2430001 and S2601501 were characterised for comparative purposes, homogeneity and stability of these two samples were not addressed.

3. METHODS

3.1 Ultraviolet/visible absorption spectra

UV/Vis absorption spectra in the region 220 – 750 nm were measured on 0.002 and 0.01% w/v solution of S2539801 in Ultrapure water. The solutions were placed in a 1cm quartz cell referenced against blank Ultrapure water. The Varian Cary 1E spectrometer was operated with a bandwidth of 1nm and a response time of 0.1 s.

Calculation of absorption coefficient, A, for a 1% solution in a 1cm cell

$$A_{1cm}^{1\%} = \frac{\text{absorbance}}{C} \quad \text{where } C = \text{solution concentration in \% (w/v).}$$

3.2 [REDACTED]

S2539801 was analysed for carbon, hydrogen, nitrogen and chlorine by Butterworth Laboratories Ltd.

Carbon, hydrogen and nitrogen analysis was carried out using a Leeman CE440 analyser. The test item was combusted in pure oxygen, producing a variety of gaseous materials: carbon dioxide from the oxidation of organic and elemental carbon and decomposition of carbonates; water vapour from the oxidation of organic hydrogen and the liberation of moisture and nitrogen and nitrogen oxides from the oxidation of organic nitrogen. The combustion products were then passed over suitable reagents in the combustion tube to ensure complete oxidation and removal of undesirable by-products, such as [REDACTED]. In the reduction tube, oxides of nitrogen were converted to molecular nitrogen and residual oxygen was removed. In the mixing volume the remaining nitrogen, carbon dioxide and water are thoroughly homogenised at precise volume, temperature and pressure before passing through to a thermal conductivity detector using chemical traps to segregate individual responses. The detector was calibrated using acetanilide.

Chlorine was determined by oxygen flask combustion and ion chromatography.

3.3 [REDACTED]

A sample was submitted to Measurement Science, which is not part of the UK GLP compliance programme, for [REDACTED]

The sample was dissolved in Ultrapure water, acidified to give a 5% nitric acid solution and analysed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The sample

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was introduced to the ICP-AES via a Low flow gem cone nebuliser with Cyclonic spray chamber. The sample was quantified by comparison of its emission intensity to that of a known standard at a wavelength characteristic to [REDACTED]

Trace metals

To determine arsenic, lead, cadmium, copper, zinc, chromium, cobalt, nickel and antimony the sample was dissolved in Ultrapure water and acidified to give a 10 % nitric acid solution. The samples were introduced into the ICP-AES by an Ultrasonic nebuliser (USN) and are quantified by comparison of their emission intensity to that of standards at wavelengths characteristic of the desired elements. Standards were spiked with [REDACTED] to match the spectral effects from the high levels of [REDACTED] present in samples.

For manganese the same sample and standard preparation procedures were used but the determination was carried out using the Low flow gem cone nebuliser with Cyclonic spray chamber.

Mercury

The sample solution prepared for trace metals analysis i.e. 10 % nitric acid was spiked with concentrated hydrochloric acid to provide a 5 % hydrochloric acid concentration. Mercury was determined by an FIAS-100, flow injection system with atomic absorption spectrometry. Samples were quantified by comparison of their emission intensity to that of known standards.

Ash

Ash was determined by heating the sample at 550 °C in a muffle furnace overnight.

3.4 Determination of [REDACTED] by ultraviolet / visible spectrometry

The aim of the method was to determine the amount of [REDACTED] present in the samples. Calibration standards in the range [REDACTED] were prepared by adding the appropriate amounts of a [REDACTED] to the chelating agent 10mM 2,2':6',2''- terpyridine, both prepared in ethanol. A calibration was constructed for concentration of [REDACTED] against absorbance at 552nm on the uv/vis spectrophotometer. The amount of [REDACTED] was calculated from its absorbance at 552nm. For samples approximately 16 mg was made up to 10 mL in 10 mM 2,2':6',2''- terpyridine in ethanol. To this 0.4 mL of 0.1 M sodium ascorbate in water was added and left to react for 5 minutes to reduce any [REDACTED] present to [REDACTED]

3.5 Liquid chromatography / mass spectrometry

The aim of this method was to confirm the identity of the main component and any significant impurities present from the molecular ions formed in the ESI-MS process. The samples at a concentration of 1000 µg/mL in initial mobile phase were analysed on an 1100

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LC/MS G1946B from Agilent Technologies Ltd. The samples were separated by gradient elution. The mobile phase consisted of a mixture of A, 5 mM ammonium formate pH 3.0 and B, acetonitrile. The mobile phase composition was 19 % B at the beginning of the gradient and then linearly increased to 100 % B in 10 min. It was then held for a further 10 min before re-equilibrating at the initial conditions for 20 min. The samples were separated by means of a Luna C₈ (2) analytical column (150 x 2mm i.d., 5 µm particle size) from Phenomenex. The flow rate was 0.20 mL/min. and 10 µL of the samples, in initial mobile phase, were injected onto the column which was thermostatically held at 30°C. The MS was operated in positive electrospray ionisation (ESI) mode with gas temperature 300°C, drying gas 7.0 L/min, nebuliser gas pressure 35 psi, capillary voltage 3000 V and fragmentor voltage of 90V. The MS was operated in full scan mode over the range m/z 50 to 750.

BL1749 Batch 005 (S2430001) was also analysed by this technique for comparative purposes with the main test item (S2539801).

3.6 Determination of total volatiles by oven drying

The total volatiles content of the samples was determined by oven drying at 105 °C for approximately 16hr, recording the difference in weight.

3.7 Purity and homogeneity of catalyst by liquid chromatography / ultraviolet detection

LC/UV was used to determine purity of the samples by consideration of the percentage areas of the main component and any impurities present. Homogeneity within the bulk sample was also considered by analysis of different sub-samples. Reference materials of [REDACTED] (JK07106) and [REDACTED] (BL1749 (E284-2003)) were analysed to confirm the retention time of the main active and [REDACTED] impurity in test item S2539801.

The samples, at a concentration of 500 µg/ml in mobile phase, were analysed by reversed phase LC by injecting 10 µL onto a Waters Spherisorb S5 C₆ column held at 35°C. An isocratic mobile phase, flowing at 1.5 ml/min., consisting of 35% acetonitrile and 65% ultrapure water containing 10mM triethylamine and 10mM octanesulphonic acid sodium salt was used for the analysis. The aqueous portion was made to pH 2.5 with o-phosphoric acid before adding the acetonitrile. Detection was by UV at 260nm with visible data at 390 nm being collected for certain samples.

LC/UV profiles for samples S2539801 and S2430001 were compared.

LC/UV profiles for samples S2539801 and S2601501 were compared.

3.8 Analysis by gas chromatography/mass spectrometry

S2539801 and S2430001 were analysed as 1000 µg/mL solutions in methanol. Compounds used in the synthesis of the catalyst were also analysed, namely 2-pyridine carboxaldehyde

(99%), dimethyl 1,3-acetone dicarboxylate (98.7%) and 2-(aminomethyl) pyridine (99%), supplied by Aldrich with purity the figures in parentheses. These standards were analysed at 1000 µg/mL and as 1 % w/w spikes (nominal values) in test item solutions to help in identification and quantification purposes.

The samples were analysed on a 6890 gas chromatograph (GC) coupled to a 5973N mass selective detector (MSD) from Agilent Technologies Ltd. The carrier gas was helium at a constant flow of 1.6 ml/min. The samples were separated by a temperature gradient starting at 40°C held for one minute then increasing to 300°C at a rate of 20°C/min. It was then held for a further 5 min before re-equilibrating at the initial temperature. The samples were separated by means of a DB5-MS analytical column (30 m x 0.25 mm i.d., 0.25 µm film thickness) from Agilent. 1 µL of the samples were injected onto the column in split injection mode (100:1) with the injector held at 250°C. The MS was operated in electron impact ionisation mode over the range 25 to 400 amu.

The samples were also run on an MD800 MS with Trace 2000 GC from ThermoFinnigan, using a SolGelWax 30 m x 0.25 mm x 0.25 µm column (SGE). Conditions used were similar to above, however the maximum temperature of the gradient in this case was 250 °C.

3.9 Quantitation of [REDACTED] using spiked addition

To give a % w/w value for the [REDACTED] impurity in S2539801 the sample was analysed using the method of spiked addition using the [REDACTED] reference standard described earlier. Methodology already detailed in Section 3.7 was used for this purpose with [REDACTED] spiked at 0, 1, 5, 10 and 20 % w/w in 1000 µg/mL solutions of S2539801. Analysis was compared in both LC/MS and LC/UV (260 nm) detection modes.

3.10 Determination of volatiles by headspace GC/MS

Headspace/GC-MS conditions were applied to solutions of S2539801 and analyte spiked solutions for the determination of formaldehyde. The 37% formaldehyde standard contains 10-15% methanol, which has similar fragment ions m/z 29 and 30 to formaldehyde. The DB-WAX-ETR GC column resolved formaldehyde from methanol.

Standards were analysed at 0, 18.5, 37, 74 and 185 µg of formaldehyde (supplied by Aldrich as a 37 % solution) in 10 mL headspace vials containing 5 mL of solution. Formaldehyde was also analysed at 18.5 and 185 µg, spiked in a solution containing 125 mg of S2539801. For approximate calculation purposes the amount of methanol present in the standard was assumed to be half the level of formaldehyde (~18.5%)

Sealed headspace vials were placed on a CombiPAL autosampler, agitated and heated at 80 °C for 10 min before 1000 µL aliquots were sampled using a gas tight syringe, heated to 105 °C. Each aliquot was injected via the Finnigan 8000 series split/splitless injector (heated to 160 °C), at 3 psi constant pressure and a 20:1 split onto a 50m x 0.32mm DB-WAX-ETR column film thickness 1µm. The Finnigan 8000 GC oven, held at an initial temperature of 30 °C for 1 min, was then heated to 150 °C at 20 °C/min.

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The MD800 in EI+ ionisation was operated in the following modes :

- i) Selected ion recording (SIR) of ions m/z 15, 28 (N_2 check ion), 29 and 30. Each with a dwell time of 0.08 s with 0.02 s inter-channel delay. Under these conditions Formaldehyde eluted at 4.46 min and methanol eluted at 6.16 min. The m/z 29 ion was used for quantitation of the formaldehyde.
- ii) Full scan from m/z 10 to 300 at 0.45 scans/s, with 0.05 s inter-scan delay.

A series of liquid and solid samples were run under different headspace incubation temperatures, (80 °C, 100 °C, 125 °C, 150 °C) and over a longer time of 20 min at 150°C, in scan mode as detailed above.

3.11 Stability of [REDACTED]

Stability of S2539801 was assessed by reanalysis using LC/MS and LC/UV with experimental conditions as described earlier.

3.12 Study dates

The study was conducted from 29th October 2003 to 12th November 2004.

3.13 Storage and retention of data

The protocol, any amendments, the raw data and final report will be placed in Datacare Business Management Systems, 3012 Heyford Park, Upper Heyford, Bucks, OX25 5HF. Datacare is not a member of the UK GLP compliance programme. The test item(s) will be archived in [REDACTED]

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4. RESULTS AND DISCUSSIONS

4.1 Ultraviolet/visible absorption spectra

For sample S2539801 the following absorption coefficients and wavelength maxima were observed.

$$A_{1cm}^{1\%} = 180 \text{ (257 nm)}$$

$$A_{1cm}^{1\%} = 25.2 \text{ (387 nm)}$$

Photobiological testing was recommended due to an absorbance of >1 being observed in the range 300 to 700nm.

4.2 [REDACTED]

The results of the [REDACTED] from Butterworth Laboratories are presented in Table 1. The [REDACTED] results from Measurement Science have also been included in the Table. From the calculations shown the experimentally derived formula of [REDACTED] is as follows:

[REDACTED]

The proposed structure is [REDACTED] (see Figure 1), which was achieved by drying to remove water before [REDACTED] was carried out¹. The experimentally derived structure is therefore consistent with the proposed structure with the addition of 1 water molecule (H₂O).

Table 1 also compares the theoretical and experimental [REDACTED] composition.

4.3 [REDACTED]

Results of [REDACTED] determinations in [REDACTED] are as follows:

[REDACTED]

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[REDACTED]

The [REDACTED] can be explained by formation of a [REDACTED] on [REDACTED] which is proposed to be the [REDACTED]. Theoretically 8.1 % w/w of [REDACTED] would give 11.6 % w/w of the [REDACTED] if this assumption was correct, which is in good agreement with the data above.

4.4 Determination of [REDACTED] by ultraviolet/visible spectroscopy

[REDACTED] was determined as follows:

[REDACTED] (S2539801) = < 0.14 % w/w

4.5 Liquid chromatography/mass spectrometry

The proposed structure of [REDACTED] main active is shown in Figure 1. As determined by positive ESI LC/MS the structure of the catalyst (main active) and [REDACTED] impurity are shown in Figures 2 and 3 respectively. In Figure 2 the structure of the catalyst shown is after replacement of chlorine with formate in solution, which occurs in the mobile phase used. The structure of the [REDACTED] shown in Figure 3 is the likely structure in the acidic mobile phase and site of protonation of the [REDACTED] could equally be at another position. Extracted ion data showed catalyst [REDACTED] (m/z 620.1), [REDACTED] (m/z 520.2) and a peak at m/z 457.2, tentatively identified as the N_2Py_2 species. These other impurities, tentatively identified from their molecular ions are displayed in Figure 4, as are typical LC/MS total ion and extracted ion chromatograms (TIC and EIC).

BL1749 Batch 005 (S2430001) was found to contain no [REDACTED] by comparison of TIC traces with [REDACTED] (S2539801).

4.6 Determination of total volatiles by oven drying

[REDACTED] contained 3.07 % w/w total volatile material. This figure appears to be in agreement with the [REDACTED] data where the addition of 1 molecule of water to the proposed structure is equivalent to 2.7 % w/w.

4.7 Purity of catalyst by liquid chromatography/ultraviolet detection

The purity of the main active (catalyst) in [REDACTED] by LC/UV at 260 nm was determined to be 97.89 %, on a peak area basis (see Table 2). The data presented in table 2 also demonstrates homogeneity of the bulk sample by replicate analysis of 3 sub-samples. From this data two main impurities were seen in the sample (see Figure 5). These were the [REDACTED] peak area 0.59 %, retention time (RT) 9.2 min and the [REDACTED] of the main active

(peak area 1.21 %, RT 2.2 min). Other unidentified small impurities were observed in the LC/UV data but individual peaks were < 0.2 % of the total area.

Reference materials of and [REDACTED] (JK07106) and [REDACTED] (BL1749 (E284-2003)) were run at the same concentrations and conditions which confirmed the retention time of the main active and [REDACTED] impurity in test item S2539801 (see Figure 5). Although unconfirmed by a retention time standard, the [REDACTED] of the main active is accepted to be the peak eluting at 2.2 min¹. The results of the comparison of LC/UV profiles of S2539801 and S2430001 are displayed in Table 3. The main difference between the two samples was the presence of the [REDACTED] at 0.6 % in S2539801 and < 0.1 % in S2430001 on an area basis.

Analysis by LC/UV of the [REDACTED] (S2601501) clearly showed that all the [REDACTED] had been removed from the sample (see Figure 6). The only peaks remaining in the sample were the [REDACTED] main active and a small peak at approximately 4.4 min. The peak at 4.4 min (0.1 % by area) was not present when the sample was reanalysed at 390 nm, i.e. not [REDACTED] containing. The identity of this peak is unknown and did not appear in any previous or subsequent analysis of S2539801 and could be considered to be an artefact of this analysis.

4.8 Analysis by gas chromatography/mass spectrometry

The results of a comparison of S2430001 and S2539801 can be clearly seen in data taken from the ThermoFinnigan MD800 analysis (Figure 7). Both sample chromatograms in the figure show very similar profiles and in each case the only peaks not present in the blank were the peaks at retention time 1.08 and 6.54 min. The mass spectra of these two peaks are also included in Figure 7. The peak at 1.08 min was identified from the library as chloromethane. The library did not correctly identify the peak at 6.54 min and the mass spectrum did not match those of any of the starting materials. The software did however identify a structure containing a pyridine ring and side chain, similar to groups contained within the catalyst structure. Both peaks were thought to be the product of thermal decomposition at the injection temperature of 250 °C (reference study KY030438 [REDACTED] decomposed at approximately 200 °C).

Data from the Agilent GC/MS confirmed that 2 pyridine carboxaldehyde was present at < 1 % w/w by spiked addition in solutions of S2539801 and S2430001. However dimethyl 1,3-acetone dicarboxylate and 2-(aminomethyl) pyridine were unable to be detected in the same solutions at the 1 % w/w level. Further method development would have been necessary to conclusively confirm the absence of these two starting materials at a known % level. However the objective of this piece of work was to confirm the similarity of S2539801 and S2430001 by this technique which was found to be the case.

4.9 Quantitation of [REDACTED] using spiked addition

[REDACTED] was determined to be 1.1 % w/w by LC/MS standard addition. The 20 % w/w [REDACTED] standard was not included in the standard addition curve due

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to a saturated MS response at this level. The curve showed good linearity excluding this standard ($R^2=0.9988$). The [REDACTED] was determined to be 0.55 % w/w using the LC/UV data at 260nm ($R^2 = 0.9892$). The MS data is considered to be the more reliable from comparison of the two correlation coefficients (R^2 values).

4.10 Determination of volatiles by headspace GC/MS

Formaldehyde was determined to be less than $<0.2 \mu\text{g}/\text{mg}$ (% w/w). The spiking standards into the matrix at detection level and 10 times the detection level gave mean recoveries of 82 and 91% respectively.

Trace amounts of methanol were found present at $<0.5 \mu\text{g}/\text{mg}$ (% w/w). This result was estimated, based on methanol present in the formaldehyde standard. In Scan mode under the under different headspace incubation temperatures a peak tentatively identified as chloromethane was identified from library fit.

4.11 Stability assessment

Analysis by LC/UV showed satisfactory stability of [REDACTED] as stored at ambient in the dark, over the duration of the study. Figure 8 demonstrates good agreement of the chromatograms as analysed at the beginning and end of the study. The area % of the main components of the sample are displayed in the figure. LC/MS confirmed the identity of the main active (m/z 634.2) and presence of [REDACTED] impurity (m/z 534.2) at the end of the study period. There was a slight increase in the catalyst [REDACTED] on storage (1.2 to 1.8 % area by LC/UV).

5. CONCLUSION

[REDACTED] (S2539801) was characterised by a variety of techniques including liquid chromatography/mass spectrometry (LC/MS), liquid chromatography/ultraviolet detection (LC/UV), total volatiles, inductively coupled plasma/ atomic emission spectroscopy (ICP/AES) and [REDACTED]. The key results are summarised as follows:

From the [REDACTED] the experimentally derived formula was [REDACTED]. This was consistent with the proposed structure with the addition of 1 water molecule. The total volatile material, determined at 3.07 % w/w, accounted for the presence of this water (theoretically 2.7 % w/w). The purity of the main active (catalyst) by LC/UV at 260 nm was determined to be 97.89 %, on a peak area basis. The homogeneity of the bulk sample, by replicate analysis of 3 sub-samples, was also demonstrated by this technique. Two main impurities were seen in the LC/UV data, which were the [REDACTED] (peak area 0.59 %), and the [REDACTED] of the main active (peak area 1.21 %). Structures of the main components of the sample were confirmed by LC/MS molecular ions. Other smaller impurities were tentatively identified by LC/MS. The amount of [REDACTED] present, determined by spiked addition LC/MS, was 1 % w/w which gave good agreement with the % area data. The sample was demonstrated to be stable over the course of the study, as stored at ambient temperature in the dark, by reanalysis using LC/MS and LC/UV.

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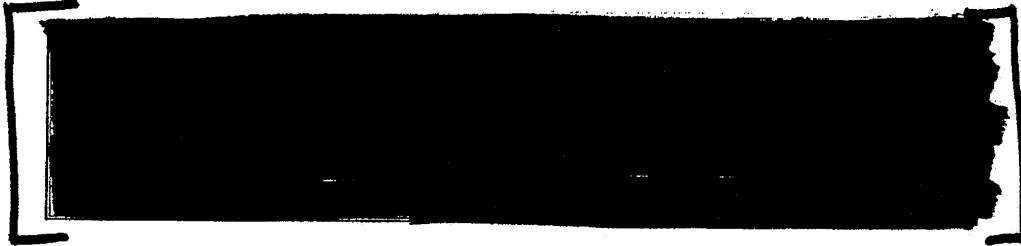
6. REFERENCES

1. Personal Communication. Jan Koek, Unilever Research, Vlaardingen.

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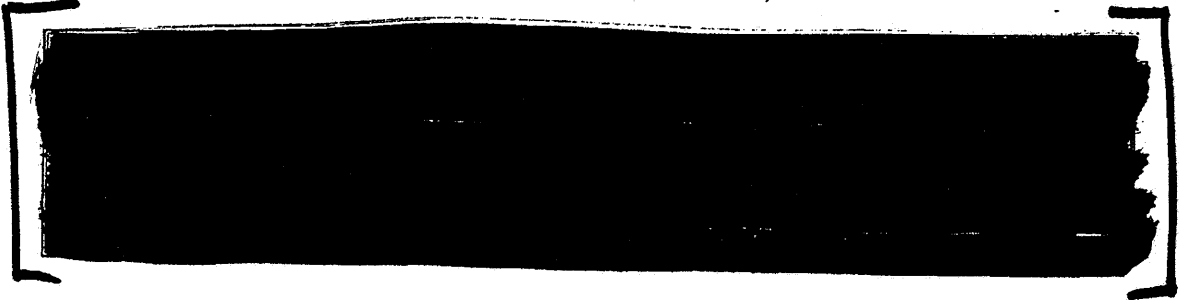
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TABLE 1. [REDACTED] OF S2539801

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* Oxygen by difference

** Data from Measurement Science (all other data Butterworth Laboratories Ltd)

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TABLE 2. LC/UV (260 nm) PURITY AND HOMOGENEITY (S2539801)

SOLUTION/SUB-SAMPLE	REPLICATES	PEAK AREA % (APPROXIMATE RETENTION TIME 2.2 MIN)	PEAK AREA % CATALYST (APPROXIMATE RETENTION TIME 4.5 MIN)	PEAK AREA % (APPROXIMATE RETENTION TIME 9.2 MIN)
001 A (sol 1)	1	1.15	98.00	0.48
001 A (sol 1)	2	1.16	98.06	0.47
001 A (sol 2)	1	1.17	97.96	0.54
001 A (sol 2)	2	1.17	97.97	0.54
001 A (sol 3)	1	1.32	97.85	0.54
001 A (sol 3)	2	1.34	97.91	0.47
001 A (sol 3)	3	1.32	97.90	0.49
001E	1	1.23	97.87	0.59
001E	2	1.25	97.84	0.60
001E	3	1.23	97.82	0.65
001I	1	1.16	97.78	0.77
001I	2	1.15	97.78	0.78
001I	3	1.15	97.82	0.73
	Mean	1.21	97.89	0.59
	SD	0.07	0.09	0.11
	%CV	5.86	0.09	19.07

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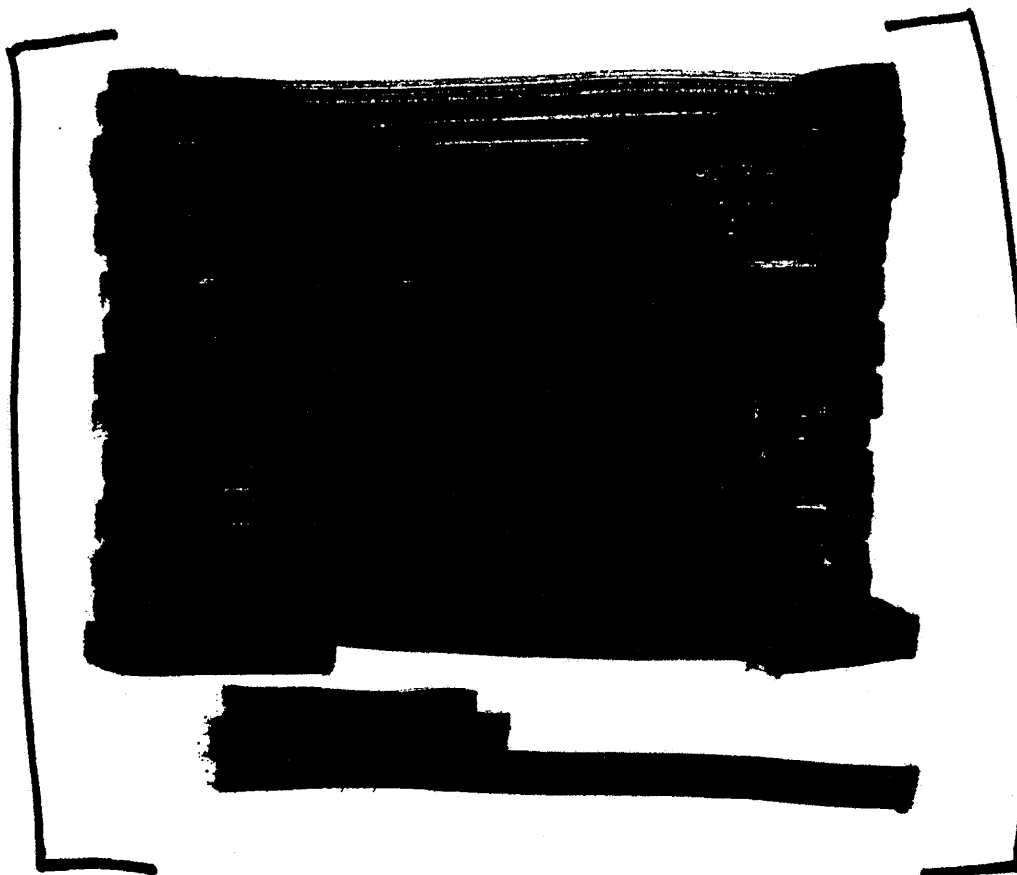
TABLE 3. COMPARISON OF S2539801 AND S2430001 BY LC/UV AT 260 NM

SAMPLE	REPLICATES	AREA % [REDACTED]	AREA % CATALYST	AREA % [REDACTED]	AREA % UNKNOWN
(Retention time (min))		(2.5)	(5.5)	(7.7)	(7.4)
[REDACTED]	A	1.228	98.124	0.648	0.000
(S2539801)	B	1.136	98.245	0.619	0.000
	C	1.197	98.105	0.599	0.000
Mean		1.187	98.158	0.622	0.000
BL1749 Batch 005	A	3.308	96.481	<0.1	0.212
(S2430001)	B	3.102	96.644	<0.1	0.254
	C	3.093	96.598	<0.1	0.252
Mean		3.167	96.574	<0.1	0.240

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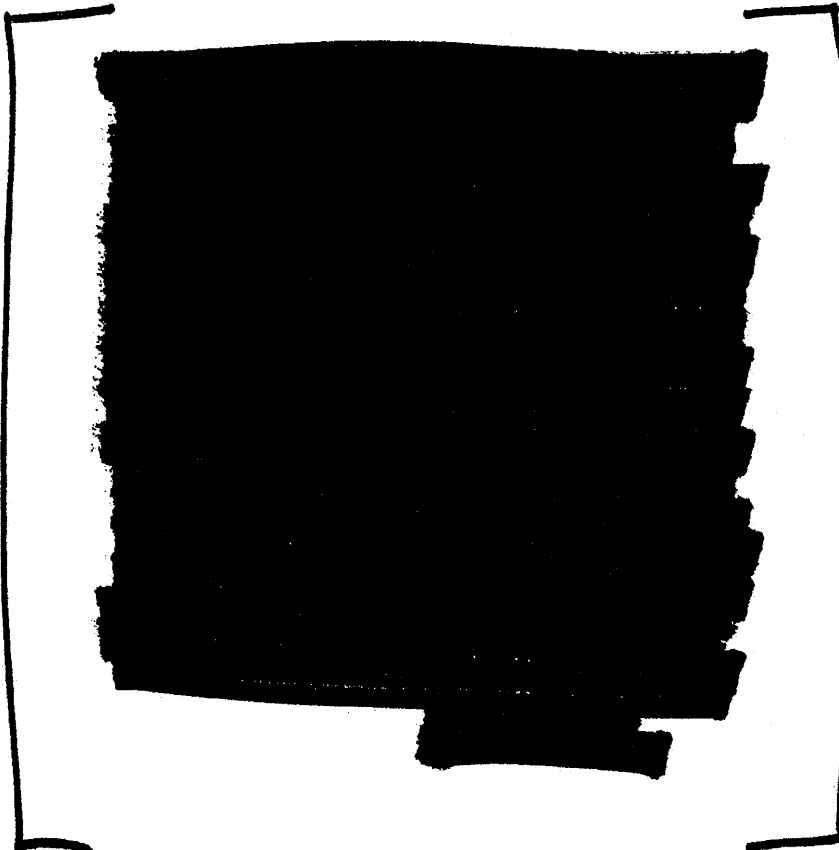
FIGURE 1. PROPOSED STRUCTURE AND FORMULA OF [REDACTED]
(S2539801)



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FIGURE 2. STRUCTURE OF CATALYST ION BY LC/MS



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FIGURE 3. STRUCTURE OF LIGAND ION BY LC/MS

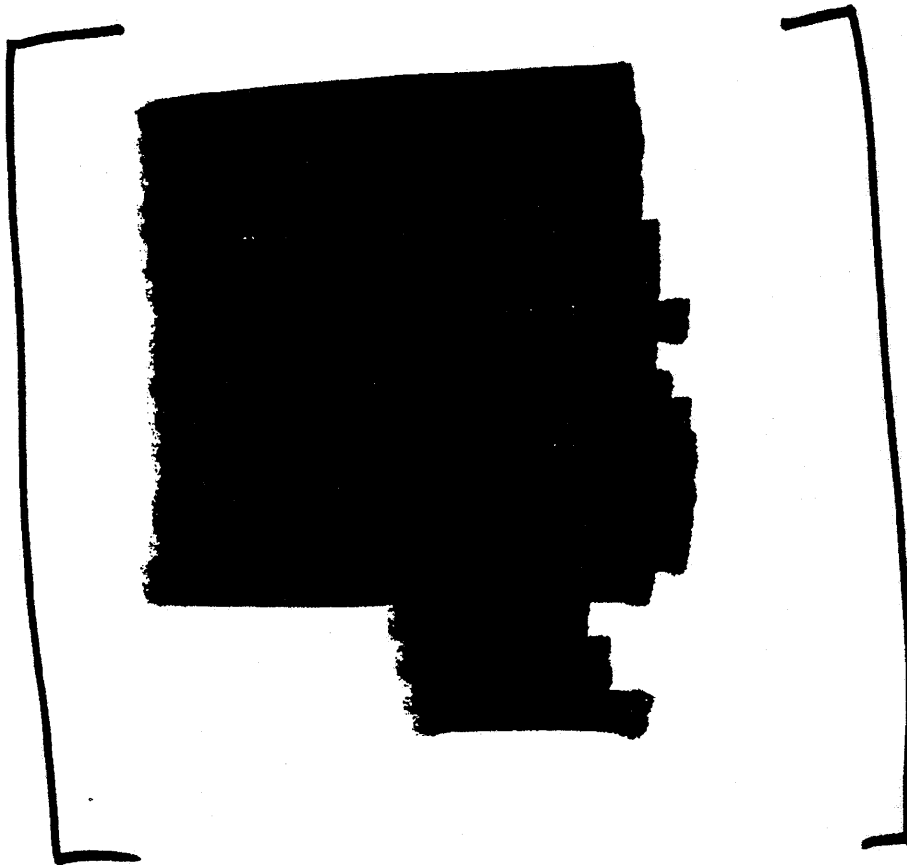
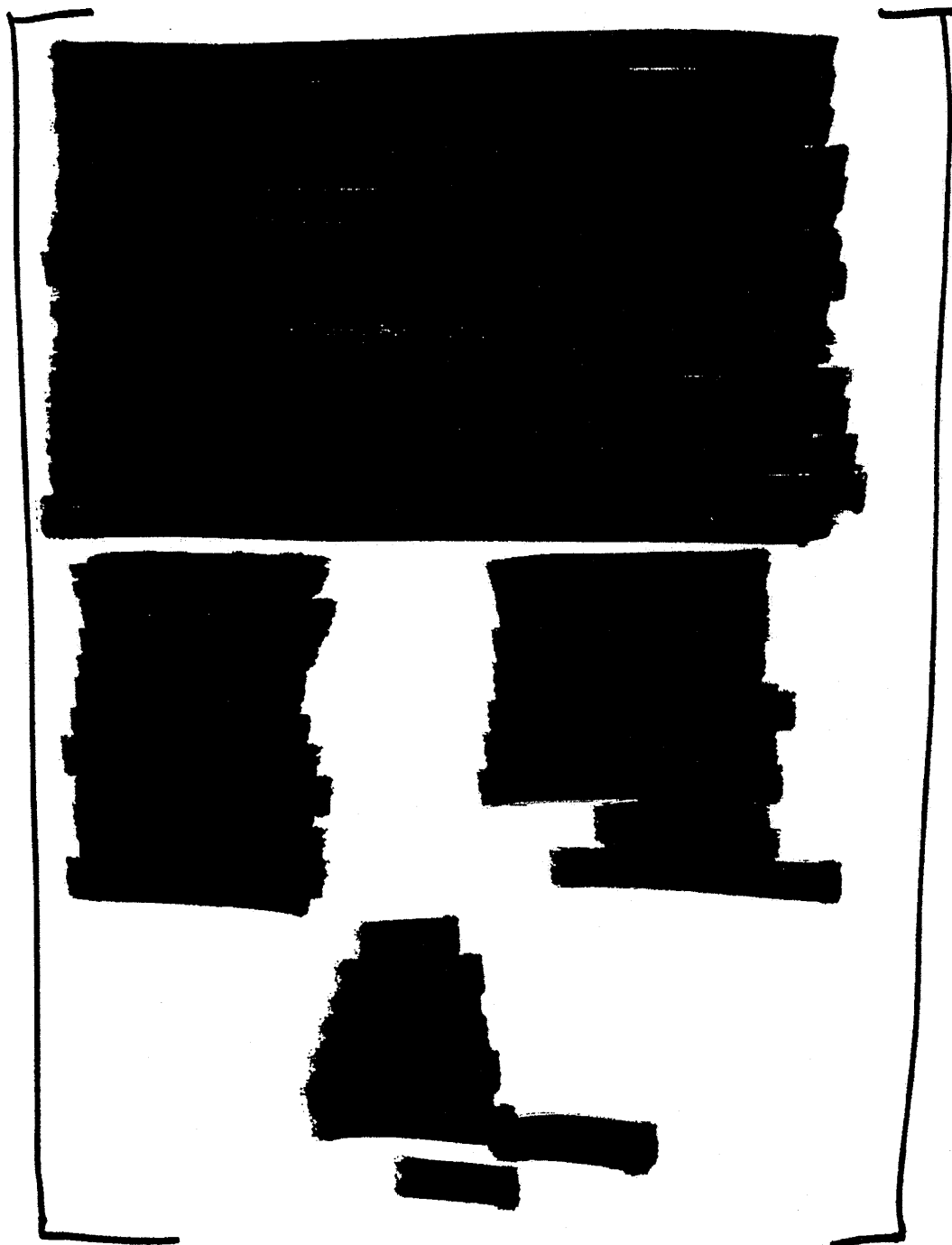


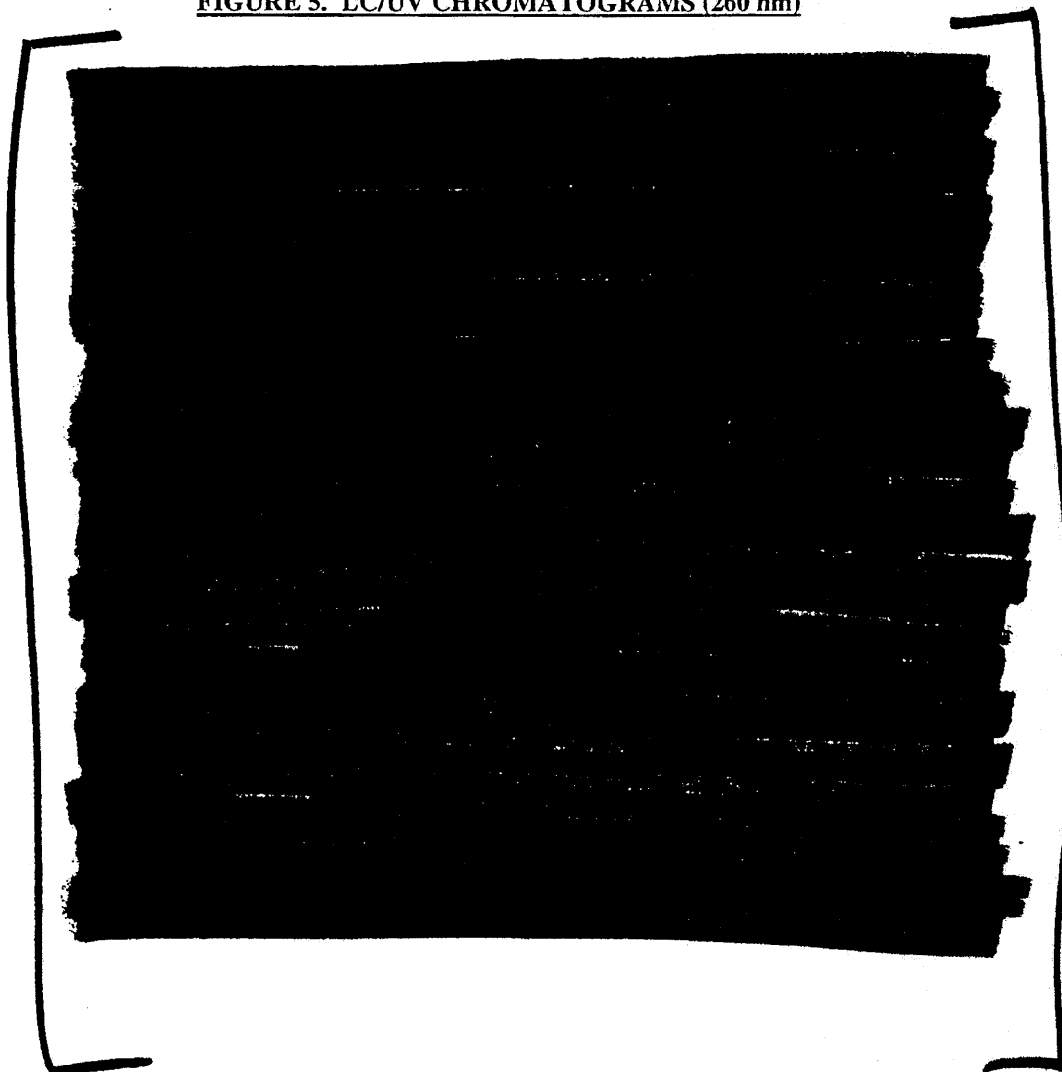
FIGURE 4. TYPICAL TIC AND EICs BY LC/MS WITH TENTATIVE IDENTIFICATION OF IMPURITY STRUCTURES.



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FIGURE 5. LC/UV CHROMATOGRAMS (260 nm)



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**FIGURE 6. THE [REDACTED] SAMPLE [REDACTED]
(S2601501) AND COMPARISON WITH S2539801**

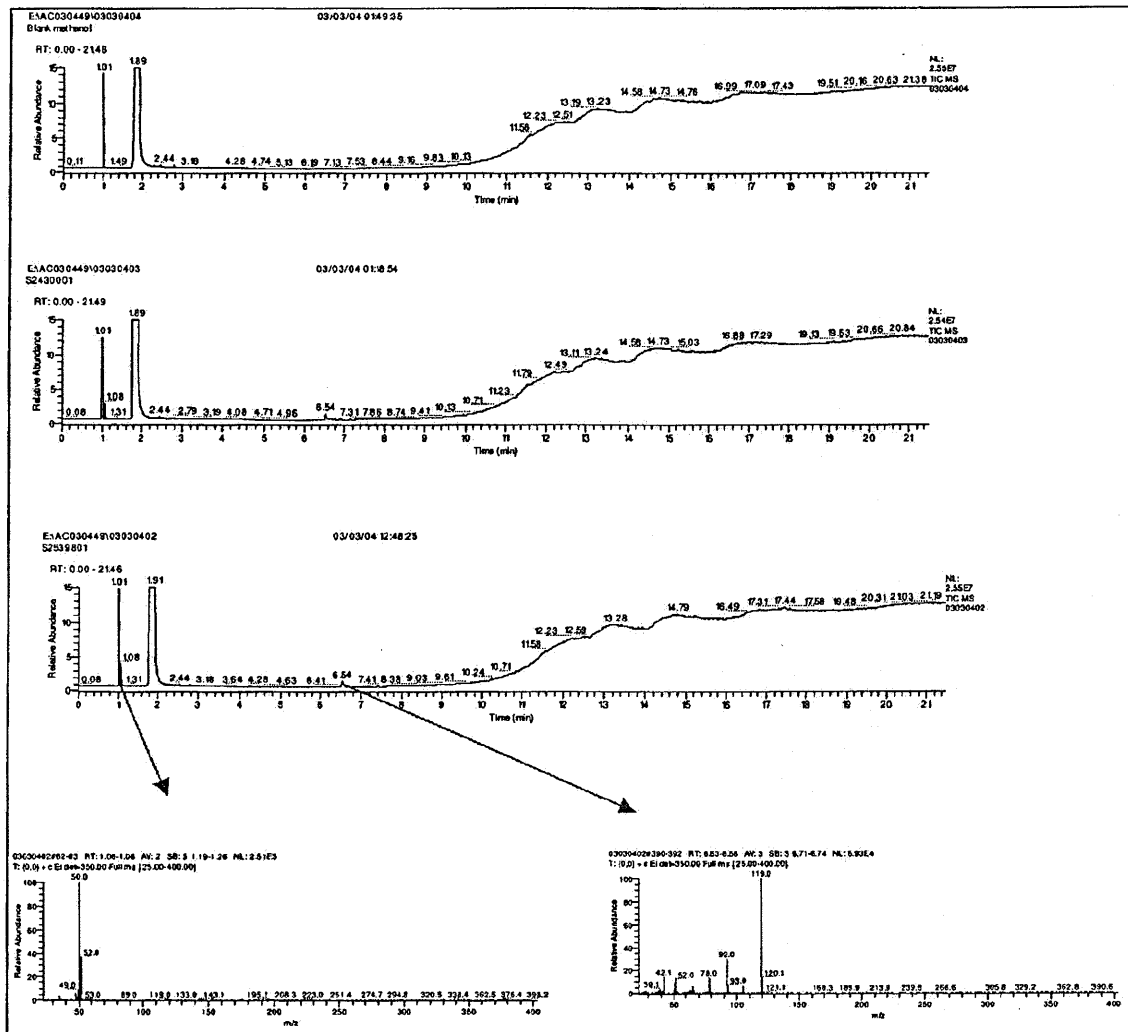


For S2539801 the figures displayed in brackets are from the analysis done at the time of comparison with S2601501. The main figures are from the initial (homogeneity) analysis.

Study AC030449

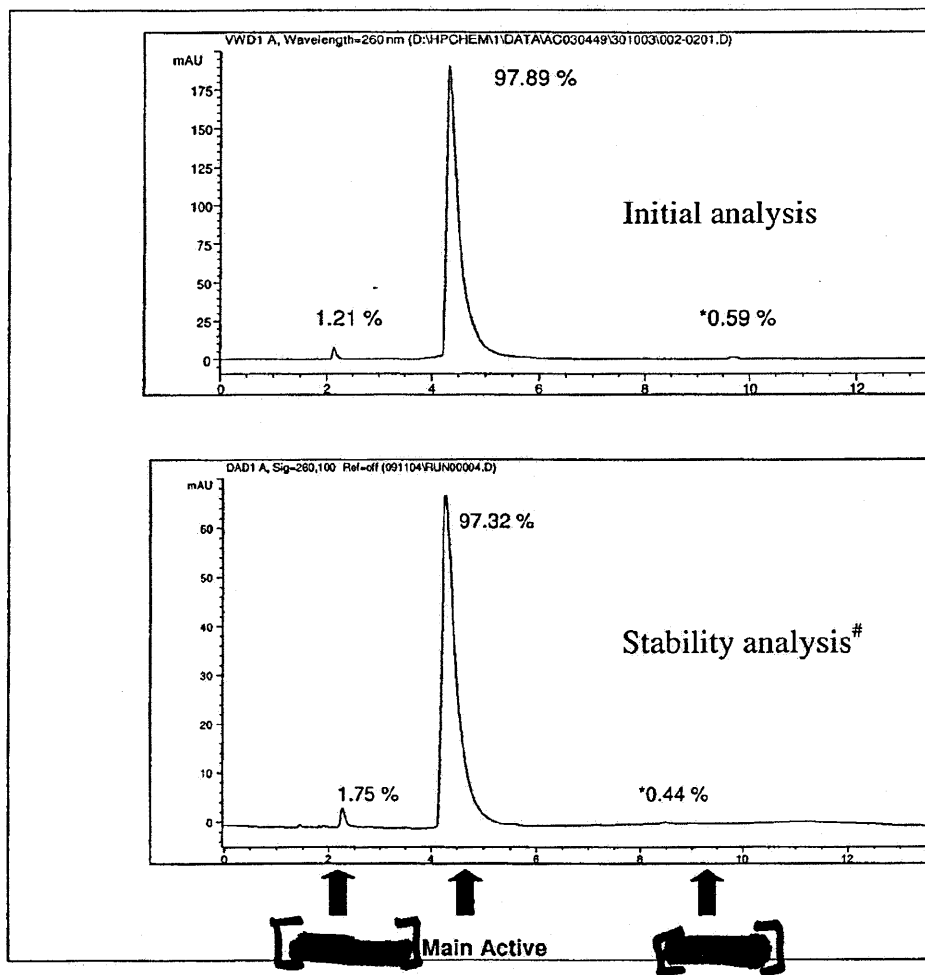
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FIGURE 7. GC/MS COMPARISON OF S2430001 AND S2539801 AND MASS SPECTRA



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FIGURE 8. STABILITY OF [REDACTED] BY LC/UV

#1 year stability

DISTRIBUTION LIST

Study AC030449

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STUDY REPORT

[REDACTED]
HOMOGENEITY AND STABILITY IN WATER

Study AH030452

AUTHORS : SEAN O'CONNOR & CHRIS SPARHAM (STUDY DIRECTOR)

[REDACTED]
This report must not be circulated further, copied, or destroyed without
reference to the Reports Administrator [REDACTED]

[REDACTED]
Date : February 2004

STUDY INFORMATION

Study title : [REDACTED] Homogeneity and Stability in Water

Study number : AH030452

Study location :



STUDY DATES

Date protocol signed : 10 November 2003

Experimental period : 18 November – 02 December 2003

Archiving (raw data) : Datacare Business Management Systems
3012 Heyford Park
Upper Heyford
Bucks OX25 5HF

Archiving (Test Material)



STUDY PERSONNEL

Study Director : Chris Sparham

Chemical Analyst : Sean O'Connor

Scientific Reviewer : Ian Bromilow

QA : Harjit Lall

CROSS REFERENCES

Project number : 221130

AUTHORISATION STATEMENT

Study number : AH030452

Study title : [REDACTED] Homogeneity and Stability in Water

This report has been authorised for issue to the appropriate recipients.

Julia Fentem
JULIA FENTEM
GROUP HEAD, APPLIED SCIENCE AND TECHNOLOGY
[REDACTED]

24/02/04
DATE

[REDACTED]

QUALITY ASSURANCE STATEMENT

Study Number : AH030452

Study Title [REDACTED] Homogeneity and Stability in Water.

This study was conducted at [REDACTED]

Procedural inspections are not performed on individual routine studies but Process Inspections are periodically conducted according to defined Standard Operating Procedures. Facility Inspections are also periodically completed.

The dates on which the relevant inspections and audits were performed and the dates on which any findings were reported to the Study Director and to Management are given below.


Audit Type	Audit Date	Report Date
Protocol Audit	10-Nov-2003	10-Nov-2003
Study Report Audit	13-Feb-2004	13-Feb-2004
Facility Inspection	27-Nov-2003	05-Dec-2003
Process Inspection	01-Sep-2003	27-Nov-2003
<ul style="list-style-type: none"> - Measurement Procedures - HPLC 		

As far as can reasonably be established, this report has been accepted by Quality Assurance as being an accurate presentation of the raw data and findings of the study.


 H LALL

19 Feb 2004

DATE

 QUALITY ASSURANCE


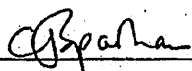
AUTHENTICATION STATEMENT

Study number : AH030452

Study title : [REDACTED] Homogeneity and Stability in Water

I, the undersigned, hereby declare that this study has been conducted under my supervision, as Study Director, in accordance with [REDACTED] policy on Good Laboratory Practice which is based on the UK Good Laboratory Practice Regulations 1999, No. 3106 and OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM/ (98)17.

I also certify that this report presents a true and accurate account of the procedures used and the results obtained.



CHRIS SPARHAM
STUDY DIRECTOR

19/2/04

DATE

[REDACTED]

[REDACTED]

HOMOGENEITY AND STABILITY IN WATER

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[REDACTED]

HOMOGENEITY AND STABILITY IN WATER

SUMMARY

[REDACTED] This study was designed to provide homogeneity and stability data in support of a toxicological study (or studies). The analysis was carried out using High Performance Liquid Chromatography with ultra violet detection at 260nm.

The data for the % nominal concentrations of the 0.5, 5 and 50 mg/mL solutions showed:

- a) "Homogeneity" was acceptable as replicate preparations all gave values within $\pm 2.1\%$ of the nominal, clearly demonstrating these were true solutions.
- b) Stability within $\pm 7.2\%$ of nominal was proven over the course of 7 days refrigerated storage, including leaving out in the laboratory on days 1, 2, 3, 6 and 7 for up to 9 hours, plus 1 day frozen storage.

"Homogeneity" ($\pm 12\%$ of the nominal) and stability ($\pm 10\%$ of the nominal) at a lower concentration of 0.08mg/ml were also proven. This lower concentration was not used for the toxicological study.

Study AH030452

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1. INTRODUCTION

[REDACTED] This study was designed to provide homogeneity and stability data in support of a toxicological study (or studies).

Study AH030452

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2. TEST MATERIAL

The sample was registered in Compound Control under the following number

Sample name :

[REDACTED]

Sample number :

S2539801

The sample was stored in dark ambient conditions prior to analysis. Characterisation, stability and homogeneity is being carried out in study number AC030449

Study AH030452

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3. METHOD

3.1 Homogeneity and stability

[REDACTED] S2539801 was prepared at concentrations covering the expected dosing levels to be used in the toxicological study. The solutions were prepared in duplicate at 0.08, 0.5, 5 and 50 mg/mL in Ultrapure water.

The samples were prepared in duplicate and analysed on day 0 to assess "homogeneity" to confirm they were true solutions. To assess stability, samples were kept refrigerated, but left out in the laboratory on days 1, 2, 3, 6 and 7 for up to 9 hours. This was to simulate what could happen during the dosing period. Analysis of 0.5, 5 and 50 mg/mL solutions took place on days 1, 3 and 7. The 0.08 mg/mL was analysed on day 0 and 7 only, as this solution was added to the study at a later date, to cover predicted dosing level changes at the CRO. The 0.5, 5 and 50 mg/mL solutions were subsequently frozen after analysis on Day 7, defrosted on Day 8 and reanalysed. This was to simulate frozen transport of the samples from the CRO to the laboratory. The main component peak area was measured by High Performance Liquid Chromatography with UV detection (HPLC/UV) as described below.

The solutions were analysed by reversed phase HPLC by injecting 10 µL onto a Waters Spherisorb S5 C₆ (250 x 4.6mm) column held at 35°C. An isocratic mobile phase, flowing at 1.5 mL/min., consisting of 35% acetonitrile and 65% ultrapure water containing 10 mM triethylamine and 10 mM octanesulphonic acid sodium salt was used for the analysis. The aqueous portion was made to pH 2.5 with o-phosphoric acid before adding the acetonitrile. Reagents wherever possible were HPLC Grade, AR Grade or equivalent. Detection was by UV at 260 nm. Calibration standards, prepared in Ultrapure water, were run at concentrations of 0.1, 0.5 and 1 mg/mL. The 5 and 50 mg/mL samples were diluted, 10 and 100-fold respectively, with Ultrapure water before analysis. The calibration standards were made fresh on each day of analysis.

3.2 Study dates

The study was conducted from 18th November 2003 to 2nd December 2003.

3.3 Storage and retention of data

The protocol, any amendments, the raw data and final report will be placed in Datacare Business Management Systems. Datacare is not a member of the GLP compliance programme. The test material will be archived in [REDACTED]

4. RESULTS AND DISCUSSION

Homogeneity and stability

Refer to Table 1. for full experimental results.

The data for the % nominal concentrations of the 0.5, 5 and 50 mg/mL solutions showed:

- b) "Homogeneity" was acceptable as replicate preparations all gave values within $\pm 2.1\%$ of the nominal, clearly demonstrating these were true solutions
- b) Stability was proven over the course of 7 days refrigerated storage, including leaving out in the laboratory on days 1, 2, 3, 6 and 7 for up to 9 hours, plus 1 day frozen storage.

"Homogeneity" ($\pm 12\%$ of the nominal) and stability ($\pm 10\%$ of the nominal) at a lower concentration of 0.08mg/ml were also proven. This lower concentration was not used for the toxicological study. The error reported on Day 8 for one of the 5 mg/mL solutions was an analytical error, but was not considered significant as all the other samples gave the expected results.

Study AH030452

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5. CONCLUSION

The data for the % nominal concentrations of the 0.5, 5 and 50 mg/mL solutions showed:

- c) "Homogeneity" was acceptable as replicate preparations all gave values within $\pm 2.1\%$ of the nominal, clearly demonstrating these were true solutions.
- b) Stability within $\pm 7.2\%$ of nominal was proven over the course of 7 days refrigerated storage, including leaving out in the laboratory on days 1, 2, 3, 6 and 7 for up to 9 hours, plus 1 day frozen storage.

"Homogeneity" ($\pm 12\%$ of the nominal) and stability ($\pm 10\%$ of the nominal) at a lower concentration of 0.08mg/ml were also proven. This lower concentration was not used for the toxicological study

TABLE 1. HOMOGENEITY AND STABILITY OF [REDACTED] (S2539801) IN ULTRAPURE WATER

Replicate	Sample Nominal Concentration (mg/ml)	Day 0		Day 1		Day 3	
		Measured concentration (mg/ml)	% Nominal	Measured concentration (mg/ml)	% Nominal	Measured concentration (mg/ml)	% Nominal
A	0.080	0.089	112	not analysed	not analysed	not analysed	not analysed
B	0.078	0.087	112	not analysed	not analysed	not analysed	not analysed
A	0.504	0.494	97.9	0.491	97.4	0.502	99.6
B	0.508	0.502	98.8	0.493	97.0	0.501	98.7
A	5.11	5.06	99.1	5.04	98.7	5.08	99.4
B	5.00	5.01	100	4.95	98.9	4.97	99.4
A	50.4	51.2	102	50.2	99.6	50.5	100
B	50.4	49.9	99.1	49.6	98.5	50.3	99.8

Replicate	Sample Nominal Concentration (mg/ml)	Day 7		Day 8/frozen	
		Measured concentration (mg/ml)	% Nominal	Measured concentration (mg/ml)	% Nominal
A	0.080	0.088	109	not analysed	not analysed
B	0.078	0.086	110	not analysed	not analysed
A	0.504	0.502	99.6	0.498	98.9
B	0.508	0.505	99.4	0.500	98.4
A	5.11	5.10	99.8	error	error
B	5.00	5.04	101	4.64	92.8
A	50.4	50.4	100	49.4	98.2
B	50.4	49.5	98.3	48.6	96.5

DISTRIBUTION LIST

Study AH030452

Archive
C. Sparham
M. York
I. Bromilow

STUDY REPORT

[REDACTED]
**HOMOGENEITY AND STABILITY IN
ECOTOXICOLOGY TESTING MEDIA**

Study AH030453

AUTHORS : SEAN O'CONNOR & CHRIS SPARHAM (STUDY DIRECTOR)

[REDACTED]
This report must not be circulated further, copied, or destroyed without
reference to the Reports Administrator [REDACTED]

[REDACTED]
Date : April 2004

STUDY INFORMATION

Study title : [REDACTED] Homogeneity and stability in
ecotoxicology testing media

Study number : AH030453

Study location : [REDACTED]

STUDY DATES

Date protocol signed : 15 December 2003

Experimental period : 16 December 2003 – 13 February 2004

ARCHIVING

Data & Report : Datacare Business Management Systems
3012 Heyford Park
Upper Heyford
Bucks OX25 5HF

Test Material : [REDACTED]

STUDY PERSONNEL

Study Director : Chris Sparham

Chemical Analyst : Sean O'Connor

Media Preparation : Environment Protection Department

Scientific Reviewer : Ian Bromilow

QA : Harjit Lall

CROSS REFERENCES

Project number : 221130

AUTHORISATION STATEMENT

Study number : AH030453
Study title : [REDACTED] Homogeneity and stability in
ecotoxicology testing media

This report has been authorised for issue to the appropriate recipients.

Julia Fentem
JULIA FENTEM
GROUP HEAD, APPLIED SCIENCE AND TECHNOLOGY
[REDACTED]

26.04.04
DATE

[REDACTED]

QUALITY ASSURANCE STATEMENT

Study Number: AH030453

Study Title: [REDACTED] Homogeneity and Stability in Ecotoxicology Testing Media.

This study was conducted at [REDACTED]

The following inspections and audits were conducted on the study. The dates on which they were performed and the dates on which any findings were reported to the Study Director and to Management are given below.

Audit Type	Audit Date	Report Date
Protocol Audit	12-Dec-2003	12-Dec-2003
Study Report Audit	06-Apr-2004	08-Apr-2004
Facility Inspection	27-Nov-2003	05-Dec-2003
Process Inspection	05-Jan-2004	19-Mar-2004
- Measurement Procedures - Wt/Vol		
- LC/MS		

As far as can reasonably be established, this report has been accepted by Quality Assurance as being an accurate presentation of the raw data and findings of the study.


H LALL20 April 2004
DATEQUALITY ASSURANCE
[REDACTED]

AUTHENTICATION STATEMENT

Study number : AH030453
Study title : [REDACTED] Homogeneity and Stability in
Ecotoxicology Testing Media

I, the undersigned, hereby declare that this study has been conducted under my supervision, as Study Director, in accordance with [REDACTED] policy on Good Laboratory Practice which is based on the UK Good Laboratory Practice Regulations 1999, No. 3106 and OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM/ (98)17.

I also certify that this report presents a true and accurate account of the procedures used and the results obtained.

Chris Sparham

CHRIS SPARHAM
STUDY DIRECTOR

21/4/04

DATE

[REDACTED]

[REDACTED]

HOMOGENEITY AND STABILITY IN
ECOTOXICOLOGY TESTING MEDIA

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[REDACTED]
HOMOGENEITY AND STABILITY IN
ECOTOXICOLOGY TESTING MEDIA

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[REDACTED]

HOMOGENEITY AND STABILITY IN
ECOTOXICOLOGY TESTING MEDIA

SUMMARY

[REDACTED] This study was designed to provide homogeneity and stability data in support of proposed ecotoxicological studies. [REDACTED] concentrations were measured by liquid chromatography/mass spectrometry (LC/MS). Dilution of samples from the relevant media into mobile phase was carried out to stabilise the samples, by lowering the pH.

Achieved concentration and homogeneity for [REDACTED] (S2539801) at concentrations of 10 and 125 mg/L were confirmed in ElenDt media (both clean and dirty). 24 hr stability for the two sets of solutions was assessed, under simulated test conditions. In clean media measured concentrations had dropped to 66.9 and 81.1 % of the nominal, respectively. This was due to alkaline hydrolysis of the principal active in [REDACTED] at the pH of the media used. Daily renewal of solutions during the course of the daphnia test was recommended.


Achieved concentration and homogeneity for [REDACTED] (S2539801) at a concentration of 125 mg/L was confirmed in "fish study" media. 24 hr stability of the solution was assessed, under simulated test conditions. Measured concentration had dropped to 90.2 % of the nominal. Daily renewal of solutions during the course of the study was again recommended. Frozen storage of aliquots of samples taken at 0 and 24hr was shown to have no adverse effect on the measured concentration. However, for the proposed contract study it was recommended that transport and analysis of samples be achieved within a 48hr period (immediate freezing and frozen transport).

Method development for [REDACTED] (S2539801) at concentrations of 30 and 300 mg/L in Isomedia containing algae was satisfactorily carried out. Full method validation, homogeneity and stability for the algal study was carried out as part of the range finder test in [REDACTED] study EAL030423.

Study AH030453

Page 2 of 11

1. INTRODUCTION

 This study was designed to provide homogeneity and stability data in support of proposed ecotoxicological studies.

Study AH030453

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2. TEST MATERIAL

The sample was registered in Compound Control under the following number

Sample name :

[REDACTED]

Sample number :

S2539801

The sample was stored in dark ambient conditions prior to analysis. Characterisation, stability and homogeneity is being carried out in study number AC030449

3. METHOD

3.1 Homogeneity and stability of [REDACTED] in Elendt media

[REDACTED] was prepared at concentrations at the top and the bottom of the test concentration range to be used in the daphnia study. "Clean" and "dirty" Elendt media was supplied by the Environment Protection department. Concentrations were prepared at 10mg/L and 125 mg/L in both media types and were made in duplicate.

At 0hr the samples were diluted 10 fold in mobile phase in duplicate and analysed to assess homogeneity. To assess stability, analysis was repeated after 24 hours of storage under simulated test conditions i.e. between 18 and 22 \pm 1 $^{\circ}$ C in a mixture of light and dark. The samples were analysed as described below.

[REDACTED] (S2539801) concentrations were measured by liquid chromatography/mass spectrometry (LC/MS). The solutions were analysed by injecting 10 μ L onto a Luna C₈ 5 μ m (150 x 2.0mm) column held at 30 $^{\circ}$ C. An isocratic mobile phase, flowing at 0.2 mL/min., consisting of 81% 5mM ammonium formate pH 3.0 and 19% acetonitrile was used for the analysis. Reagents were HPLC Grade or equivalent. The mass spectrometer was operated in positive electrospray ionisation (ESI) mode, with gas temperature 250 $^{\circ}$ C, gas flow 11.0 L/min, nebuliser pressure 30 psi and capillary voltage 3000V. Quantitation of [REDACTED] was carried out using single ion monitoring (SIM) for m/z 634.2. The calibration stock solution of [REDACTED] was prepared in Ultrapure water at 1000 mg/L (stable for 1 week in the fridge). Further dilutions in calibration standard preparation were carried out in mobile phase. The calibration standards were made fresh on each day of analysis from the stock solution. Calibration standards were run at concentrations of 0.1, 1, 10 and 15 mg/L.

3.2 Homogeneity and Stability of [REDACTED] in "fish study" media

[REDACTED] was prepared at concentrations to be used in the contract fish study. The solutions were prepared in duplicate at 125 mg/L in media prepared by the Environment Protection department.

At 0hr the samples were diluted 10 fold in mobile phase in duplicate and analysed to assess homogeneity. To assess frozen storage of these samples, i.e. to simulate what would be necessary to get the samples from the contract laboratory to [REDACTED] aliquots of the sample were immediately frozen and then analysed at timepoints, 48, 96 and 168 hr. The bulk samples were maintained at simulated test conditions (between 18 and 22 \pm 1 $^{\circ}$ C in a mixture of light and dark) for 24hr before reanalysis. Frozen storage of aliquots of the 24hr samples was tested after freezing for 72 and 144hr.

The main component peak area was measured by LC/MS as described in Section 3.1. Calibration standards were run at 6.25, 12.5 and 25 mg/L for this part of the study.

Study AH030453

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3.3 Method development for [REDACTED] in Isomedia containing algae

Only preliminary method development was carried out in this study. Full method validation, homogeneity and stability for the algal study was carried out as part of the range finder test in [REDACTED] study EAL030423. [REDACTED] was prepared in duplicate at 30 and 300 mg/L in Isomedia and the same concentration in Isomedia with Algae, as supplied by the Environment Protection department. The samples were passed through a 0.45µm PTFE syringe filter before dilution 10 fold (10mg/L) and 25 fold (300 mg/L) in mobile phase. Samples were measured at 0hr in both Isomedia and Isomedia containing algae to check the filtration step. To assess stability, the samples were left on the bench and aliquots were taken at 24, 48 and 72 hr. The main component peak area was measured by LC/MS as described in Section 3.1. Calibration standards were run at 1.5, 3, 12 and 15 mg/L for this part of the study.

3.4 Study dates

The study was conducted from 16th December 2003 to 13 February 2004.

3.5 Storage and retention of data

The protocol, any amendments, the raw data and final report will be placed in Datacare Business Management Systems. Datacare is not a member of the UK GLP compliance programme. The test material will be archived in [REDACTED]

Study AH030453

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4. RESULTS AND DISCUSSION

4.1 Homogeneity and stability of [REDACTED] in Elendt media

Refer to Table 1. for full experimental results.

The data for the % nominal concentrations of the 10 and 125 mg/L in clean Elendt media solutions showed:

- a) Homogeneity was acceptable as replicate preparations at 0hr gave values within $\pm 8\%$ of the nominal, clearly demonstrating these were true solutions. Satisfactory achieved concentration was also demonstrated.
- b) As expected, due to pH of the media, the solutions were not stable over 24 hr test conditions storage. Results of 66.9 and 81.1 % of nominal concentration were obtained for the 10 and 125 mg/L solutions respectively.

The same concentration solutions were also analysed in dirty Elendt media, to ensure the analytical method could still be used to quantify the principal active in the test substance in the presence of any matrix interferences. This data was seen to be comparable with the clean media. In fact the dirty media afforded more stability with the 10 mg/L test solution (85.9% of nominal after 24hr).

4.2 Homogeneity and stability of [REDACTED] in "fish study" media

Refer to Table 2. for full experimental results.

Single point averaged calibrations using the standard checks at 12.5 mg/L were used to calculate data. These standards were run bracketing samples to assess the accuracy of the calibration. The average of initial and closing calibrations was not found to give the most accurate results due to the LC/MS response decreasing over the course of a run.

The data for the % nominal concentration of the 125 mg/L solutions showed:

- a) Homogeneity was acceptable as replicate preparations at 0hr all gave values within $\pm 5.1\%$ of the nominal, clearly demonstrating these were true solutions. The mean % of nominal concentration was measured at 96.0, demonstrating satisfactory achieved concentration.
- b) Stability after 24 hr under test conditions gave a mean % nominal concentration of 90.2, showing a slight drop in concentration from 0hr values.
- b) Stability of the 0 hr samples was proven for 168 hr (7 days) frozen storage with concentrations being measured $\pm 10.5\%$ of the nominal concentration after this time

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with a mean value of 96.2 % of nominal. The 24hr ambient samples were also assessed for frozen stability. Mean % nominal concentration had dropped to 83.5 after 144 hr (6 days) frozen storage, a mean drop of 6.7% from the original measurement.

4.3 Method development of [REDACTED] in Isomedia containing algae

Refer to Table 3. for full experimental results.

The methodology developed within this study was assessed in the measurement of the main active in the test item in the presence of algae, after incorporation of a filtration step. The data for the % nominal concentration of the 30 and 300 mg/L solutions containing algae showed 0 hr values of 85.4 and 107 respectively. Solutions in Isomedia only at the same concentrations gave % nominal values of 93.9 and 96.9. Stability was acceptable at the higher level over the course of 72 hr of storage on the laboratory bench with the % nominal concentration being 74.1. Stability at 30 mg/L was poor with the mean % nominal concentration dropping to 18.1 over the same time period.

Full method validation, homogeneity and stability for the algal study was carried out as part of the range finder test in [REDACTED] study EAL030423. Proper conditions for algal growth were not simulated during this laboratory bench set up.

5. CONCLUSION

Achieved concentration and homogeneity for [REDACTED] (S2539801) at concentrations of 10 and 125 mg/L were confirmed in Elendt media (both clean and dirty). 24 hr stability for the two solutions was assessed, under simulated test conditions. Measured concentrations had dropped to 66.9 and 81.1 % of the nominal, respectively. This was due to alkaline hydrolysis of the principal active in [REDACTED] at the pH of the media used. Daily renewal of solutions during the course of the daphnia test was therefore recommended.

Achieved concentration, homogeneity for [REDACTED] (S2539801) at a concentration of 125 mg/L was confirmed in "fish study" media. 24 hr stability of the solution was assessed, under simulated test conditions. Measured concentration had dropped to 90.2 % of the nominal and daily renewal of solutions during the course of the study was recommended. Frozen storage of aliquots of samples was found to have no adverse effect on the measured concentration. However for the proposed contract study transport and analysis of samples within a 48 hr period was recommended. (immediate freezing and frozen transport)

Method development for [REDACTED] (S2539801) at concentrations of 30 and 300 mg/L in Isomedia containing algae was satisfactorily carried out. Full method validation, homogeneity and stability for the algal study was carried out as part of the range finder test in [REDACTED] study EAL030423.

TABLE 1. HOMOGENEITY AND STABILITY OF [REDACTED] IN ELENDT MEDIA

SOLUTION	MEDIA	REPLICATE	NOMINAL CONCENTRATION (mg/L)	0 HOURS		24 HOURS	
				Measured Concentration (mg/L)	% of Nominal Concentration	Measured Concentration (mg/L)	% of Nominal Concentration
10 mg/L A	Clean Elendt Media	1	10.4	10.8	104	7.25	69.7
		2	10.4	9.94	95.6	7.46	71.7
10 mg/L B	Clean Elendt Media	1	10.4	10.6	102	6.83	65.7
		2	10.4	9.70	93.3	6.28	60.4
10 mg/L	Clean Elendt Media	Average		10.3	98.7	6.96	66.9
125 mg/L A	Clean Elendt Media	1	128	126	98.4	106	82.8
		2	128	123	96.1	104	81.3
125 mg/L B	Clean Elendt Media	1	128	125	97.7	103	80.5
		2	128	118	92.2	102	79.7
125 mg/L	Clean Elendt Media	Average		123	96.1	104	81.1
10 mg/L A	Dirty Elendt Media	1	10.4	10.7	103	8.74	84.0
		2	10.4	9.88	95.0	9.03	86.8
10 mg/L B	Dirty Elendt Media	1	10.4	10.1	97.1	9.06	87.1
		2	10.4	10.1	97.1	8.89	85.5
10 mg/L	Dirty Elendt Media	Average		10.2	98.0	8.93	85.9
125 mg/L A	Dirty Elendt Media	1	128	123	96.1	112	87.5
		2	128	121	94.5	112	87.5
125 mg/L B	Dirty Elendt Media	1	128	120	93.8	109	85.2
		2	128	120	93.8	110	85.9
125 mg/L	Dirty Elendt Media	Average		121	94.5	111	86.5

TABLE 2. HOMOGENEITY AND STABILITY OF [REDACTED] IN "FISH STUDY" MEDIA

Solution	Nominal Concentration (mg/L)	0 hr			24 hr		
		Sample	Measured Concentration (mg/L)	% of Nominal Concentration	Sample	Measured Concentration (mg/L)	% of Nominal Concentration
125 mg/L A	125.85	Replicate 1	119	94.9	Replicate 1	116	92.3
	125.85	Replicate 2	120	95.3	Replicate 2	114	91.0
125 mg/L B	125.85	Replicate 1	122	97.2	Replicate 1	114	90.4
	125.85	Replicate 2	122	96.8	Replicate 2	110	87.2
125 mg/L		Average	121	96.0		114	90.2
				(0 hr + 48 hr frozen)	(24 hr + 72 hr frozen)		
125 mg/L A	125.85	Replicate 1	122	96.9	Replicate 1	116	91.8
	125.85	Replicate 2	118	93.8	Replicate 2	110	87.6
125 mg/L B	125.85	Replicate 1	127	101	Replicate 1	107	85.3
	125.85	Replicate 2	119	94.5	Replicate 2	107	85.0
125 mg/L		Average	121	96.4		110	87.4
				(0 hr + 96 hr frozen)	(24hr +144hr frozen)		
125 mg/L A	125.85	Replicate 1	130	103	Replicate 1	107	84.7
	125.85	Replicate 2	127	101	Replicate 2	108	86.1
125 mg/L B	125.85	Replicate 1	125	99.2	Replicate 1	102	81.2
	125.85	Replicate 2	127	101	Replicate 2	103	82.0
125 mg/L		Average	127	101		105	83.5
				(0 hr + 168 hr frozen)			
125 mg/L A	125.85	Replicate 1	131	104			
	125.85	Replicate 2	117	92.9			
125 mg/L B	125.85	Replicate 1	123	98.1			
	125.85	Replicate 2	113	89.5			
125 mg/L		Average	121	96.2			

TABLE 3. METHOD DEVELOPMENT FOR [REDACTED] IN ISOMEDIA CONTAINING ALGAE

Solution	Nominal Concentration (mg/L)	Sample	Measured Concentration (mg/L)	% of Nominal Concentration	Sample	Measured Concentration (mg/L)	% of Nominal Concentration
		0 hr			24 hr		
30 mg/L	30.0	Replicate 1	25.6	85.3	Replicate 1	18.3	60.9
30 mg/L	30.0	Replicate 2	No sample	No sample	Replicate 2	17.6	58.6
30 mg/L		Average	25.6	85.3	Average	18.0	59.8
300 mg/L	300	Replicate 1	322	107	Replicate 1	260	86.4
300 mg/L	300	Replicate 2	No sample	No sample	Replicate 2	262	87.2
300 mg/L		Average	322	107		261	86.8
		48 hr			72 hr		
30 mg/L	30.0	Replicate 1	11.3	37.5	Replicate 1	5.64	18.8
30 mg/L	30.0	Replicate 2	11.7	39.1	Replicate 2	5.25	17.5
30 mg/L		Average	11.5	38.3		5.44	18.1
300 mg/L	300	Replicate 1	260	86.7	Replicate 1	229	76.3
300 mg/L	300	Replicate 2	264	87.9	Replicate 2	216	71.9
300 mg/L		Average	262	87.3		223	74.1

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**SafePharm
Laboratories**

[REDACTED]

**DETERMINATION OF
PHYSICO-CHEMICAL PROPERTIES**

SPL PROJECT NUMBER: 1736/022

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1736-022.doc/MsOffice

[REDACTED] Study Reference Number: KY030438

QUALITY ASSURANCE REPORT

Inspection of the routine and repetitive procedures that constitute the study is process based (as defined by OECD) and is designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§ 21 April 2004	Protocol Compliance Audit
07, 13, 18 May 2004	Melting Temperature
14 May 2004	Relative Density
14, 15 June 2004	Vapour Pressure
05, 07 May 2004	Surface Tension
10, 13 May 2004	Water Solubility
12, 13, 26 May 2004	Partition Coefficient
14 June 2004	Flammability (Solids)
14, 15 June 2004	Moisture Content
14, 15 June 2004	Relative Self-Ignition Temperature for Solids
§ 08 July 2004	Draft Report Audit
§ Date of QA Signature	Final Report Audit


§ Evaluation specific to this study

S. M. Crowther DATE: 24 SEP 2004

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA


 Study Reference Number: KY030438

GLP COMPLIANCE STATEMENT

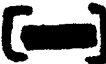
The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.


.....  DATE: 23 SEP 2004

S M Woolley AMRSC
Study Director

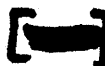
 Study Reference Number: KY030438

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[REDACTED]

**DETERMINATION OF
PHYSICO-CHEMICAL PROPERTIES**

SUMMARY

Melting/Freezing Temperature and Boiling Temperature. Decomposed prior to melting from approximately 481 K at 102.37 kPa. As the test material decomposed, no value for melting or boiling temperature could be determined, by differential scanning calorimetry, using ASTM E537-86, Methods A1 and A2 of Commission Directive 92/69/EEC.

Relative Density. 1.52 at $20.5 \pm 0.5^{\circ}\text{C}$, using a gas comparison pycnometer, Method A3 of Commission Directive 92/69/EEC.

Vapour Pressure. 5.3×10^{-6} Pa at 25°C , Method A4 of Commission Directive 92/69/EEC.

Surface Tension. 70.7 mN/m (1.00 g/l solution) at $19.5 \pm 0.5^{\circ}\text{C}$, using a ring method based on ISO 304, Method A5 of Commission Directive 92/69/EEC. The test material is considered not to be a surface-active material.

Water Solubility. 77.5 g/l of solution at $20.0 \pm 0.5^{\circ}\text{C}$, using the flask method, Method A6 of Commission Directive 92/69/EEC.

Partition Coefficient. 9.09×10^{-4} at $20.6 \pm 1^{\circ}\text{C}$, $\log_{10} P_{ow}$ -3.04, using the shake-flask method, Method A8 of Commission Directive 92/69/EEC.

Flammability (Solids). Not highly flammable as the test material did not propagate combustion over the 200 mm of the preliminary screening test in under 4 minutes, Method A10 of Commission Directive 92/69/EEC.

Explosive Properties. Based on the chemical structure of the test material it was considered unnecessary to carry out the explosive properties test according to Method A14 of Commission Directive 92/69/EEC. There are no significant functional groups that infer explosive properties. Therefore, the test result has been predicted as negative.

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Relative Self-Ignition Temperature for Solids. 202°C, Method A16 of Commission Directive 92/69/EEC.

Oxidising Properties. Based on the chemical structure of the test material it was considered unnecessary to carry out the oxidising properties according to Method A17 of Commission Directive 92/69/EEC. There are no significant functional groups that infer oxidising properties. Therefore, the test result has been predicted as negative.

[REDACTED] Study Reference Number: KY030438

[REDACTED]

**DETERMINATION OF
PHYSICO-CHEMICAL PROPERTIES**

1. INTRODUCTION

Physico-chemical properties of the test material have been determined.

Methods employed complied with those specified in Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Testing was conducted between 05 May 2004 and 23 June 2004.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[REDACTED]
[REDACTED]	:	S2539801
Description	:	[REDACTED]
Date received	:	06 April 2004
Storage conditions	:	room temperature, in the dark

The identity, purity and stability of the test material (test item) is being addressed by the Sponsor in a GLP compliant study ([REDACTED] Study AC030449).

3. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal. The remaining test material (test item) will be returned to the Sponsor. Archiving of test materials (test items) is the responsibility of the Sponsor.

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4. MELTING/FREEZING TEMPERATURE AND BOILING TEMPERATURE

4.1 Method

The determination was carried out by differential scanning calorimetry (DSC) using the procedure specified in ASTM E537-86, Methods A1 and A2 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

4.1.1 Procedure

Sample

Aliquots (see following table) of test material were placed in pierced aluminium crucibles.

Table 4.1

Determination	Mass Taken (g)
1	0.0050
2	0.0053

Analysis

The DSC parameters were as follows:

Calorimeter : MettlerToledo DSC12E
Temperature program : initial: 25°C
rate: 5°C/min
final: 360°C
Atmosphere : air (static)

Calibration

The temperature accuracy of the DSC was assessed using an indium reference standard (purity*99.999%). The melting temperature was determined to be 156.7°C and within the defined tolerance ($156.6 \pm 0.5^\circ\text{C}$). The DSC was therefore considered acceptable for use.

* Value quoted by supplier

4.1.2 Calculation

Measured temperatures were converted from °C to K using Equation 4.1.

Equation 4.1

$$T = t + 273.15$$

where:

T = temperature (K)
t = temperature (°C)

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4.2 Results

Thermograms and thermographic data for Determinations 1 and 2 are shown in Figure 4.1 and Figure 4.2 and in the following tables respectively.

Figure 4.1 Thermogram – Determination 1

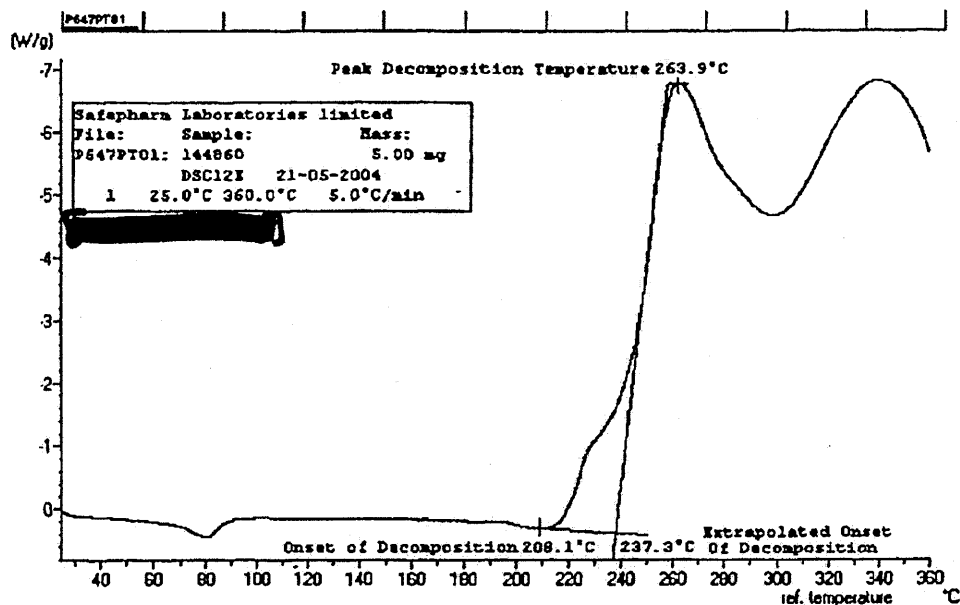


Table 4.2 Thermographic Data – Determination 1

Thermal Event	Interpretation	Temperature	
		°C	K
Rising baseline	Onset of decomposition	208.1	481
-	Extrapolated onset of decomposition	237.3	510
Exotherm	Peak decomposition temperature	263.9	537

Atmospheric pressure: 102.37 kPa

[REDACTED] Study Reference Number: KY030438

Figure 4.2 Thermogram – Determination 2

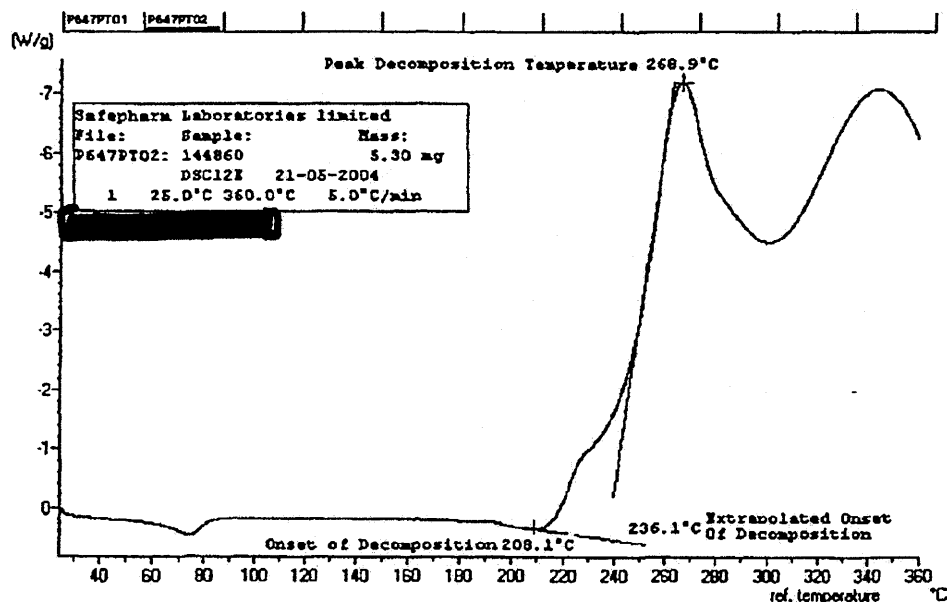


Table 4.3 Thermographic Data – Determination 2

Thermal Event	Interpretation	Temperature	
		°C	K
Rising baseline	Onset of decomposition	208.1	481
-	Extrapolated onset of decomposition	236.1	509
Exotherm	Peak decomposition temperature	268.9	542

Atmospheric pressure: 102.37 kPa

4.3 Discussion

As the test material decomposed prior to melting, no values for melting or boiling temperature could be determined. An assessment of boiling temperature is not required if the test material decomposes prior to melting.

As a result of the low rate of enthalpy change during decomposition, the onset temperature can only be approximated.


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Similar thermographic profiles were obtained using air and nitrogen atmospheres except with the nitrogen atmosphere the response was reduced. This indicated that the observed decomposition in both determinations was partially thermal and partially oxidative.

Using a metal block technique for additional information, decomposition was evident from approximately 190°C, 463K, supporting the interpretation of the DSC determinations.

4.4 Conclusion

The test material has been determined to decompose prior to melting from approximately 481K at 102.37 kPa. As the test material decomposed, no values for melting or boiling temperature could be determined.

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5. RELATIVE DENSITY

5.1 Method

The relative density was determined using a gas comparison pycnometer, Method A3 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

5.1.1 Procedure

Testing was carried out using a Quantachrome MVP-2 gas comparison pycnometer.

5.1.1.1 Calibration

A stainless steel test ball of known volume was used to calibrate the instrument prior to measurement of the test sample.

5.1.1.2 Sample

Aliquots (see following table) of test material were weighed into the sample cell of known volume (V_C), and placed into the pycnometer.

Table 5.1

Determination	Mass Taken (g)
A	28.7749
B	26.9644


Pressure readings (P_1 and P_2) were taken after pressurising the reference cell of known volume (V_R) and then switching to the sample cell (V_C).

5.1.2 Calculation

The relative density was calculated using Equation 5.1 to Equation 5.3.

Equation 5.1

$$V = V_C - V_R [(P_1 / P_2) - 1]$$

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Equation 5.2

$$\rho = \frac{1000 m}{V}$$

Equation 5.3

$$\text{Relative density} = \frac{\rho}{\rho_{H_2O, 4^\circ C}}$$

where:

V	=	volume of test sample (cm ³)
V _C	=	volume of sample cell (149.225 cm ³)
V _R	=	volume of reference cell (90.953 cm ³)
P ₁	=	pressure reading after pressurising reference cell (V _R)
P ₂	=	pressure reading after switching to the sample cell (V _C)
ρ	=	density of test material (kg/m ³)
m	=	mass of test material taken (g)
ρ _{H₂O, 4°C}	=	density of water at 4°C (999.97 kg/m ³)

[REDACTED] Study Reference Number: KY030438

5.2 Results

5.2.1 Calibration

The pressure readings (P_1 and P_2) and the calculated volume for the calibration ball are shown in the following table:

Table 5.2

Determination	P_1	P_2	Volume (cm^3)	Certified Volume (cm^3)	Tolerance (cm^3)
A	17.004	8.418	56.5	56.6	± 0.5
B	17.307	8.568	56.5	56.6	± 0.5

5.2.2 Sample

The pressure readings, calculated volumes and density values obtained for the test material are shown in the following table:

Table 5.3

Determination	P_1	P_2	Volume (cm^3)	Density (kg/m^3)
A	16.995	6.985	18.883	1523.9
B	17.098	6.990	17.701	1523.3


Temperature: $20.5 \pm 0.5^\circ\text{C}$

Mean density: 1523.6 kg/m^3

Relative density: 1.52

5.3 Conclusion

The relative density of the test material has been determined to be 1.52 at $20.5 \pm 0.5^\circ\text{C}$.

 Study Reference Number: KY030438

6. VAPOUR PRESSURE

6.1 Method

The vapour pressure was determined using a vapour pressure balance system with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25 °C. Testing was conducted using Method A4 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

6.1.1 Procedure

The vapour pressure was determined using a vapour pressure balance. The temperature of the sample was controlled electronically. The mass and temperature readings were recorded automatically into a computer file.

A diagram of the cross-section of the vapour pressure balance is represented in Figure 6.1. After evacuating the system, opening the shutter above the sample oven causes the escaping vapour jet to be directed at the scale pan. The difference in mass readings with the orifice covered and uncovered is proportional to the vapour pressure at the given oven temperature.

A sequence of runs was started after a sample of test material had been under vacuum for 15 minutes. Temperature and pressure readings were taken between 100 and 110°C with a one hour dwell at 110°C between runs.


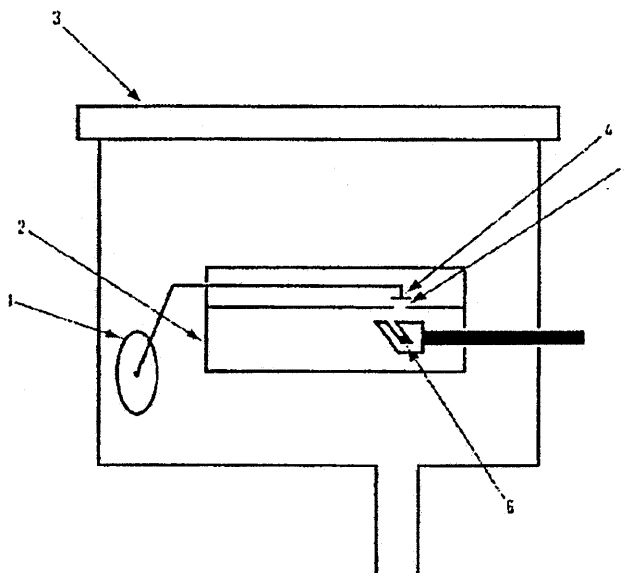
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Figure 6.1 Schematic Diagram of the Apparatus Used

- 1 Microbalance
- 2 Oven
- 3 Glass viewing panel
- 4 Balance pan
- 5 Shutter with orifice
- 6 Test sample

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6.1.2 Calculation

The vapour pressure is related to the observed mass difference by Equation 6.1.

Equation 6.1

$$V_p = \frac{\delta m \cdot g}{A}$$

where:

V_p	=	vapour pressure (Pa)
δm	=	mass difference (kg)
g	=	acceleration due to gravity (9.813 m s^{-2})
A	=	area of the orifice ($7.06858 \times 10^{-6} \text{ m}^2$)


Vapour pressure is related to temperature by Equation 6.2.

Equation 6.2

$$\text{Log}_{10} [V_p (\text{Pa})] = \frac{\text{slope}}{\text{temperature (K)}} + \text{intercept}$$

A plot of $\text{Log}_{10} V_p (\text{Pa})$ versus reciprocal temperature ($1/T(\text{K})$) therefore gives a straight line graph.

The vapour pressure of the sample was measured over a range of temperatures to enable extrapolation to 298.15 K.

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6.2 Results

Run 1

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
103	376.15	0.002658514	14.80	1.480E-08	0.020546192	-1.687268663
104	377.15	0.002651465	17.90	1.790E-08	0.024849786	-1.604677348
105	378.15	0.002644453	18.91	1.891E-08	0.026251925	-1.580838850
106	379.15	0.002637479	18.48	1.848E-08	0.025654975	-1.590828412
107	380.15	0.002630541	21.08	2.108E-08	0.029264441	-1.533659772
108	381.15	0.002623639	26.71	2.671E-08	0.037080323	-1.430856491
109	382.15	0.002616774	27.43	2.743E-08	0.038079868	-1.419304571
110	383.15	0.002609944	30.82	3.082E-08	0.042786056	-1.368697744

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 1 gives the following statistical data using an unweighted least squares treatment.


Slope -6284.192
Standard deviation in slope 628.150

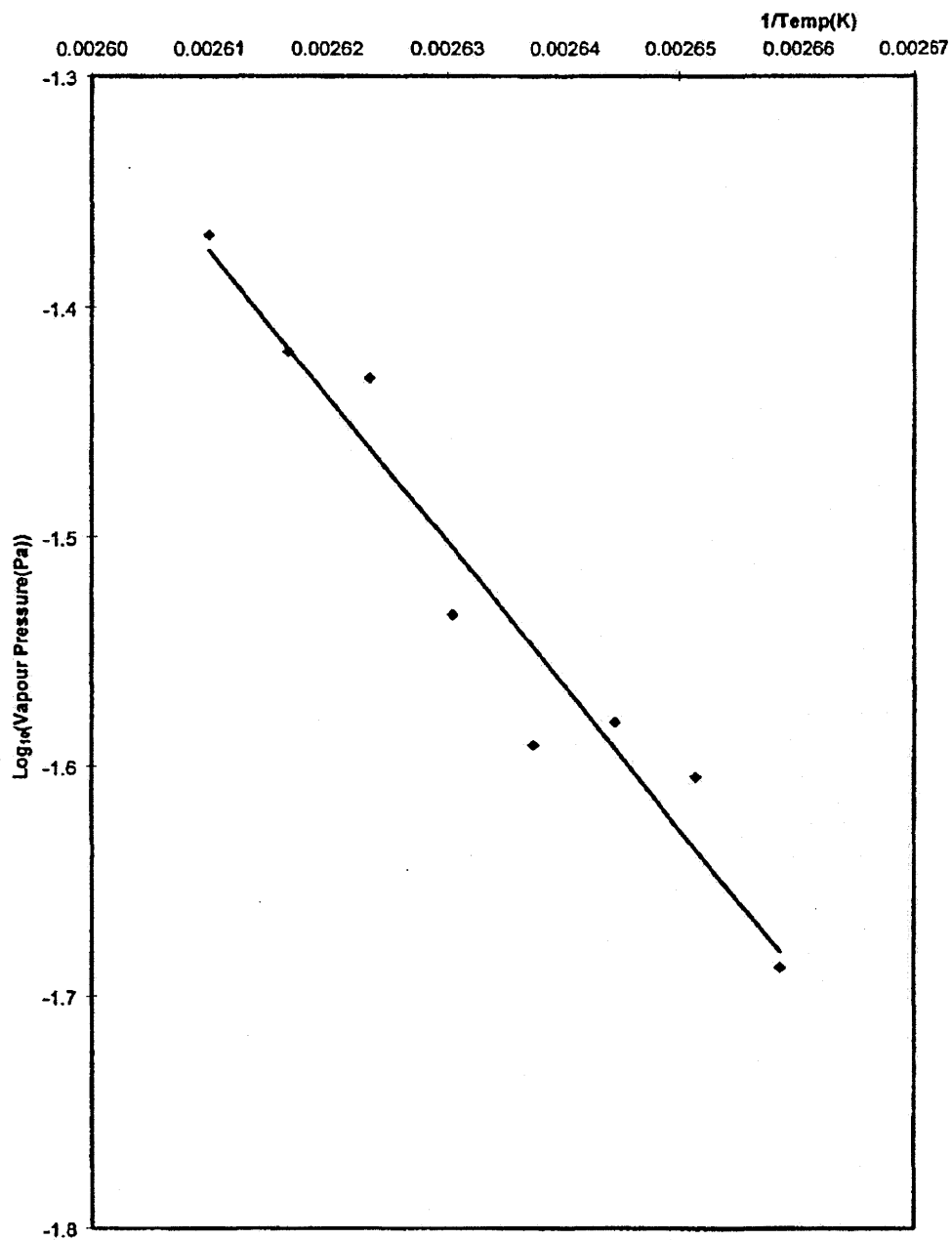
Intercept 15.026
Standard deviation in intercept 1.655

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -6284.192/\text{temp(K)} + 15.026.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -6.051.

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Run 1 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

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Run 2

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
104	377.15	0.002651465	16.10	1.610E-08	0.022350925	-1.650704503
105	378.15	0.002644453	18.77	1.877E-08	0.026057569	-1.584066106
106	379.15	0.002637479	18.98	1.898E-08	0.026349103	-1.579234171
107	380.15	0.002630541	19.78	1.978E-08	0.027459708	-1.561304091
108	381.15	0.002623639	21.22	2.122E-08	0.029458797	-1.530784999
109	382.15	0.002616774	27.50	2.750E-08	0.038177045	-1.418197685
110	383.15	0.002609944	28.87	2.887E-08	0.040078956	-1.397083595

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 2 gives the following statistical data using an unweighted least squares treatment.

Slope -5884.233

Standard deviation in slope 829.988

Intercept 13.948

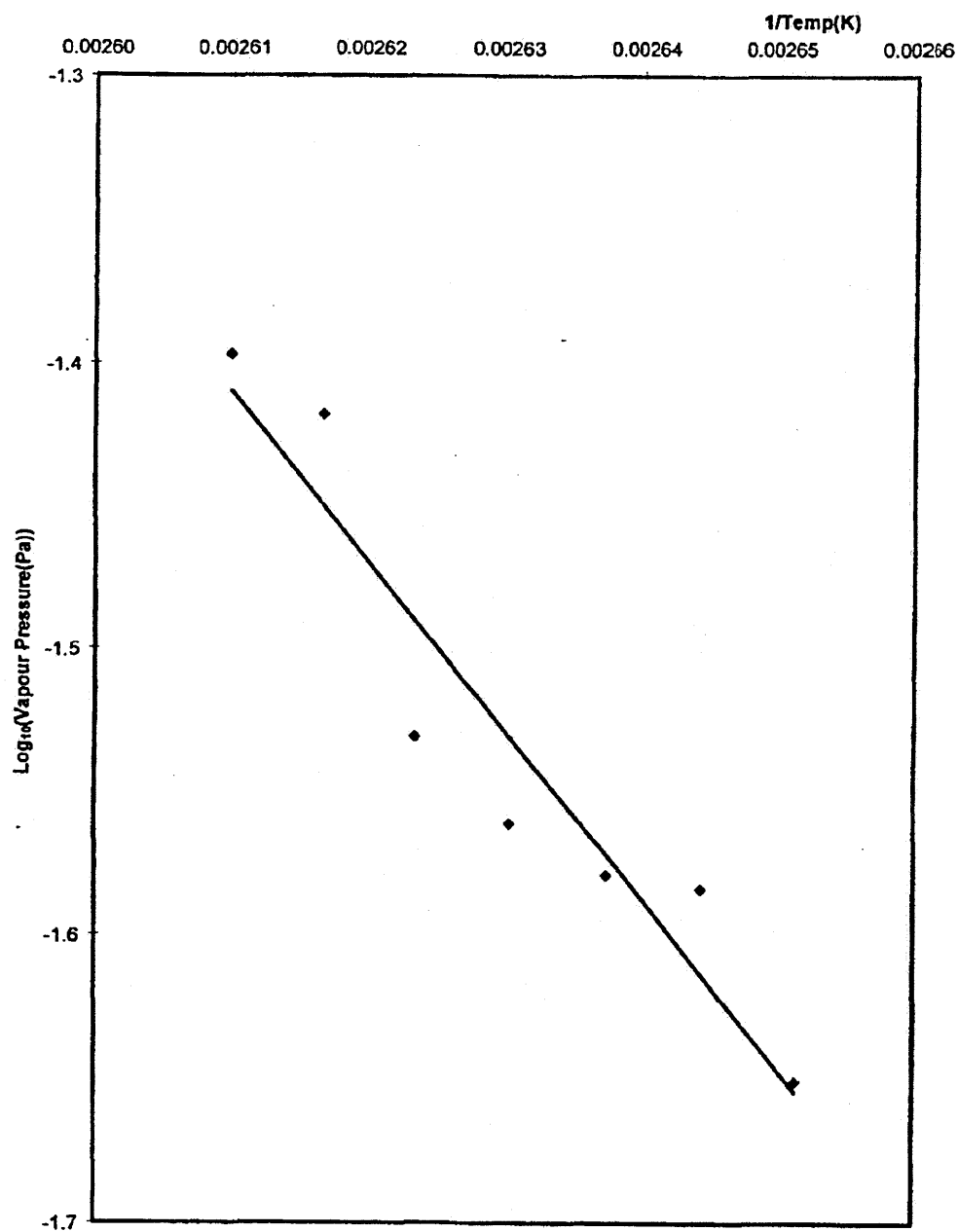
Standard deviation in intercept 2.183

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -5884.233/\text{temp(K)} + 13.948.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -5.788.

[REDACTED] Study Reference Number: KY030438

Run 2 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

[REDACTED] Study Reference Number: KY030438

Run 3

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
102	375.15	0.002665600	15.45	1.545E-08	0.021448558	-1.668601895
103	376.15	0.002658514	16.60	1.660E-08	0.023045053	-1.637422291
104	377.15	0.002651465	18.77	1.877E-08	0.026057569	-1.384066106
105	378.15	0.002644453	19.85	1.985E-08	0.027556886	-1.559769868
106	379.15	0.002637479	20.21	2.021E-08	0.028056658	-1.551964065
107	380.15	0.002630541	23.02	2.302E-08	0.031957658	-1.495425059
108	381.15	0.002623639	24.32	2.432E-08	0.033762391	-1.471566808
109	382.15	0.002616774	25.12	2.512E-08	0.034872996	-1.457510744
110	383.15	0.002609944	28.58	2.858E-08	0.039676362	-1.401468154

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 3 gives the following statistical data using an unweighted least squares treatment.

Slope -4546.416

Standard deviation in slope 225.173


Intercept 10.455

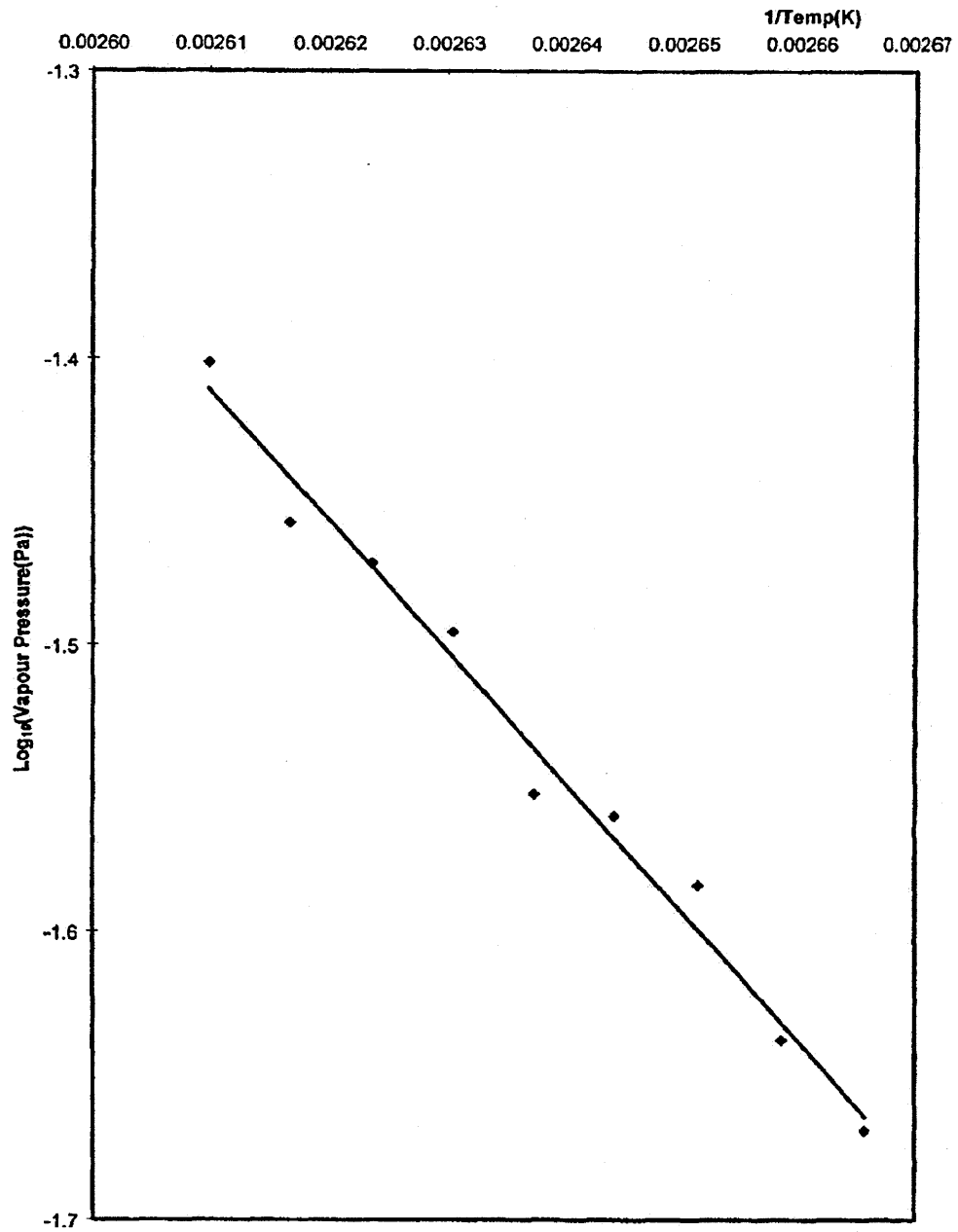
Standard deviation in intercept 0.594

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -4546.416/\text{temp(K)} + 10.455.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -4.794.

 Study Reference Number: KY030438

Run 3 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

[REDACTED] Study Reference Number: KY030438

Run 4

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
102	375.15	0.002665600	18.04	1.804E-08	0.025044142	-1.601293846
103	376.15	0.002658514	18.98	1.898E-08	0.026349103	-1.579234171
104	377.15	0.002651465	20.35	2.035E-08	0.028251014	-1.548965965
105	378.15	0.002644453	23.02	2.302E-08	0.031957658	-1.495425059
106	379.15	0.002637479	24.18	2.418E-08	0.033568035	-1.474074082
107	380.15	0.002630541	25.19	2.519E-08	0.034970174	-1.456302211
108	381.15	0.002623639	29.45	2.945E-08	0.040884145	-1.388445080
109	382.15	0.002616774	31.97	3.197E-08	0.044382551	-1.352787742
110	383.15	0.002609944	34.72	3.472E-08	0.048200255	-1.316950662

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 4 gives the following statistical data using an unweighted least squares treatment.

Slope -5213.041

Standard deviation in slope 234.951

Intercept 12.282

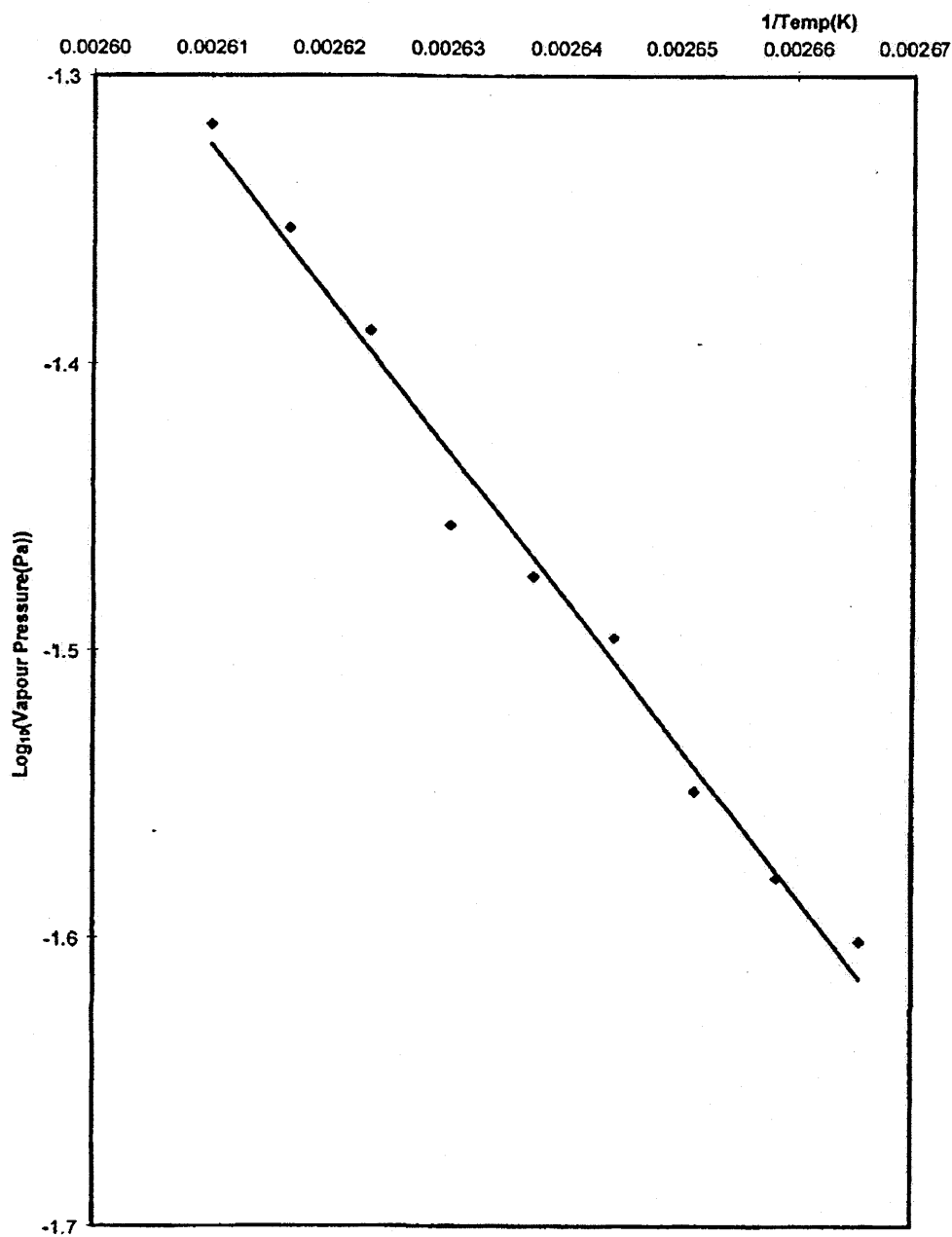
Standard deviation in intercept 0.620

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -5213.041/\text{temp(K)} + 12.282.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -5.203.

 Study Reference Number: KY030438

Run 4 - Graph of Log₁₀ Vapour Pressure vs Reciprocal Temperature

[REDACTED] Study Reference Number: KY030438

Run 5

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
101	374.15	0.002672725	18.40	1.840E-08	0.025543914	-1.592712556
102	375.15	0.002665600	18.98	1.898E-08	0.026349103	-1.579234171
103	376.15	0.002658514	17.54	1.754E-08	0.024350014	-1.613500790
104	377.15	0.002651465	23.17	2.317E-08	0.032165896	-1.492604345
105	378.15	0.002644453	23.82	2.382E-08	0.033068263	-1.480588622
106	379.15	0.002637479	25.62	2.562E-08	0.035567124	-1.448951253
107	380.15	0.002630541	23.82	2.382E-08	0.033068263	-1.480588622
108	381.15	0.002623639	32.98	3.298E-08	0.045784689	-1.339279727
109	382.15	0.002616774	31.76	3.176E-08	0.044091017	-1.355649885
110	383.15	0.002609944	33.99	3.399E-08	0.047186828	-1.326179214

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 5 gives the following statistical data using an unweighted least squares treatment.

Slope -4692.019
Standard deviation in slope 591.229

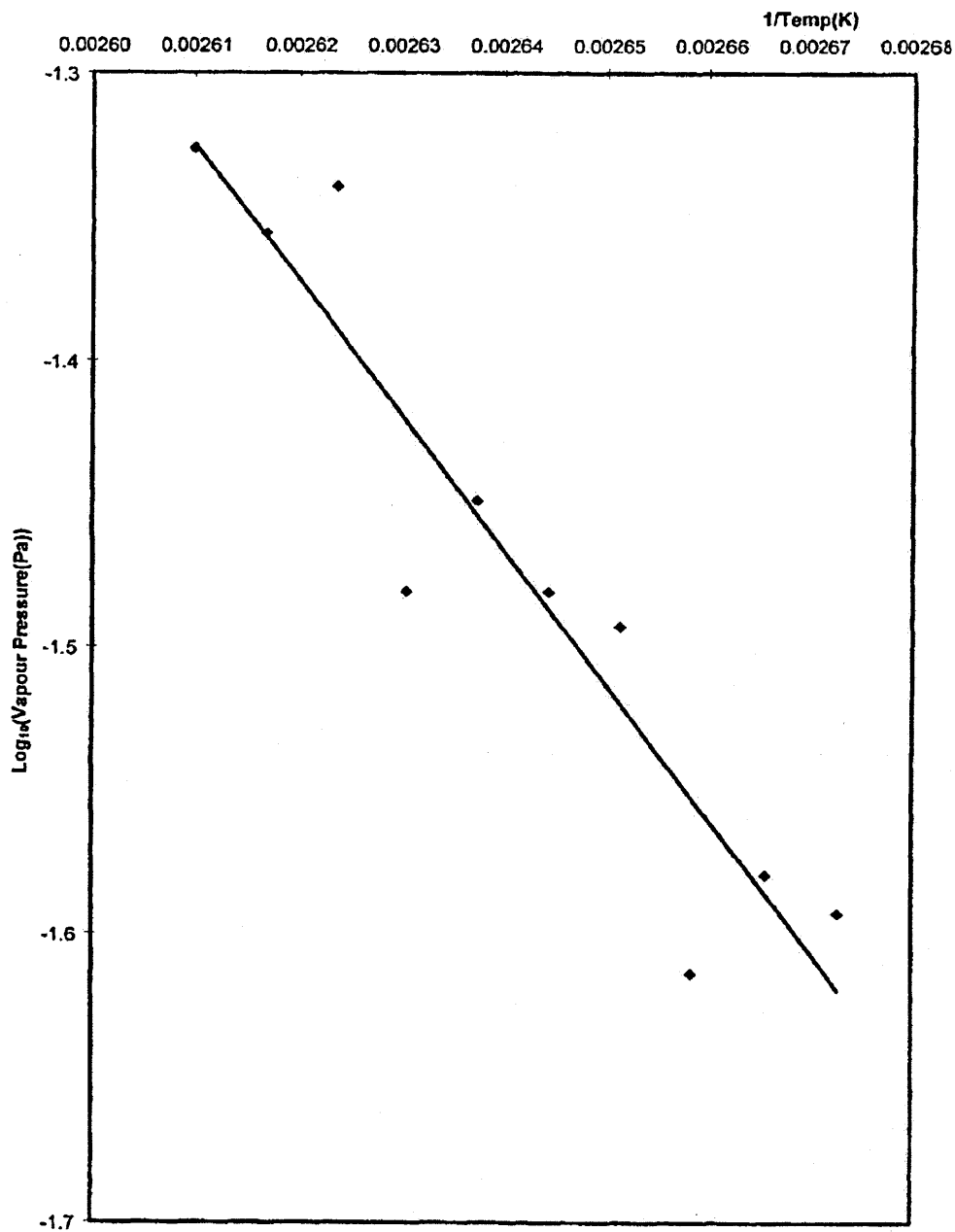
Intercept 10.921
Standard deviation in intercept 1.562

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -4692.019/\text{temp(K)} + 10.921.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -4.816.

 Study Reference Number: KY030438

Run 5 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

[REDACTED] Study Reference Number: KY030438

Run 6

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
102	375.15	0.002665600	19.05	1.905E-08	0.026446281	-1.577635399
103	376.15	0.002658514	19.49	1.949E-08	0.027057113	-1.567718540
104	377.15	0.002651465	19.56	1.956E-08	0.027154291	-1.566161528
105	378.15	0.002644453	23.60	2.360E-08	0.032762846	-1.484618376
106	379.15	0.002637479	22.52	2.252E-08	0.031263530	-1.504961993
107	380.15	0.002630541	26.63	2.663E-08	0.036969263	-1.432159212
108	381.15	0.002623639	29.74	2.974E-08	0.041286739	-1.384189415
109	382.15	0.002616774	32.41	3.241E-08	0.044993383	-1.346851348
110	383.15	0.002609944	33.78	3.378E-08	0.046895294	-1.328870733

A plot of Log₁₀ (vapour pressure (Pa)) versus reciprocal temperature (1/T(K)) for Run 6 gives the following statistical data using an unweighted least squares treatment.

Slope -4965.505

Standard deviation in slope 440.466


Intercept 11.631

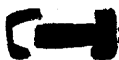
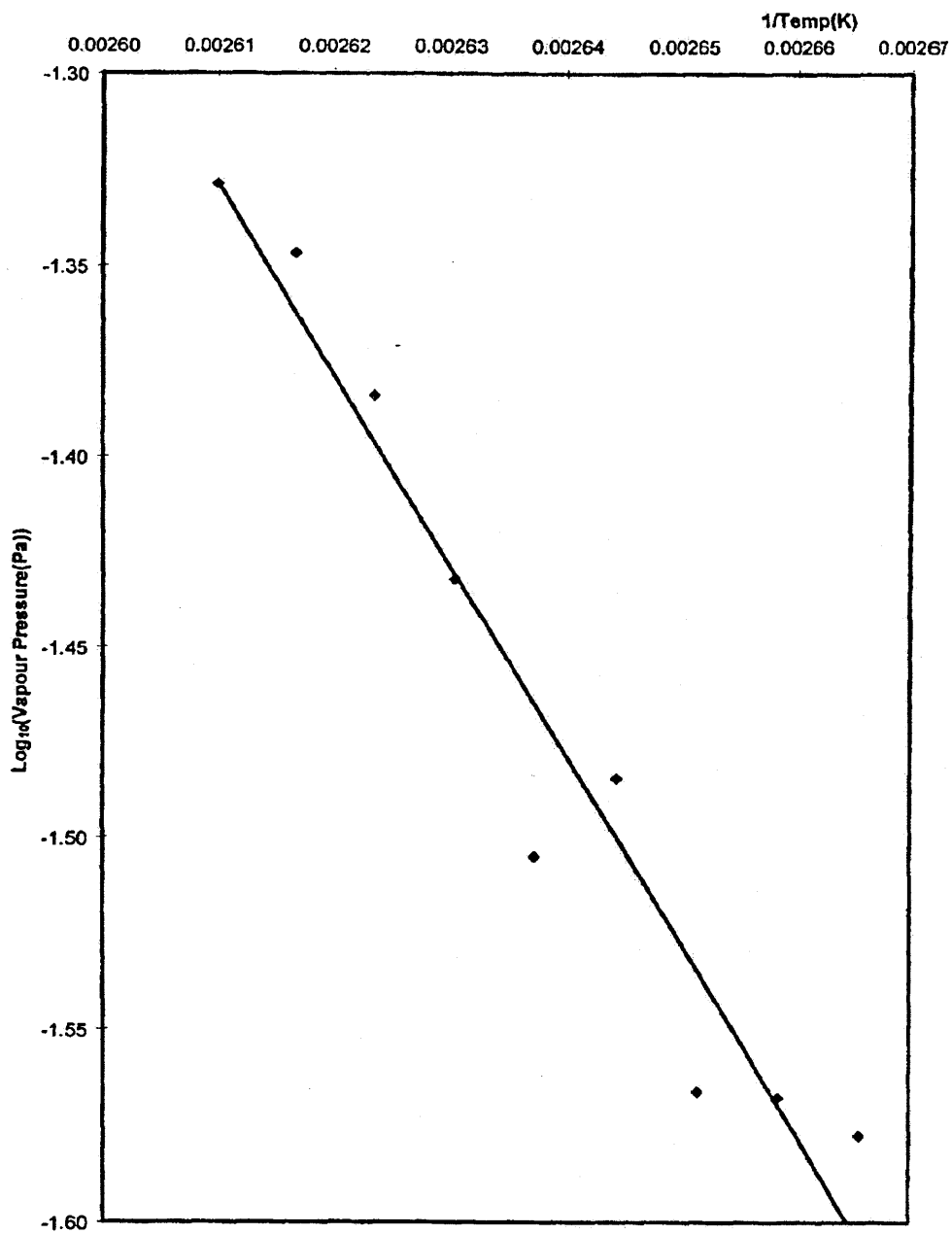
Standard deviation in intercept 1.162

The results obtained indicate the following vapour pressure relationship:

$$\text{Log}_{10} (\text{Vp (Pa)}) = -4965.505/\text{temp(K)} + 11.631.$$

The above yields a vapour pressure (Pa) at 298.15 K with a common logarithm of -5.023.

 Study Reference Number: KY030438

Run 6 - Graph of \log_{10} Vapour Pressure vs Reciprocal Temperature

Study Reference Number: KY030438

Summary of Results

Run	Log ₁₀ [Vp(25°C)]
1	-6.051
2	-5.788
3	-4.794
4	-5.203
5	-4.816
6	-5.023
Mean	-5.279
Vapour Pressure	5.3×10^{-6} Pa

6.3 Discussion

It was evident that there was a poor correlation both within each run and between runs. This was due to the low mass difference readings observed, being close to noise level. There is however, within each run, a consistent general increase of vapour pressure with temperature and so it was considered inappropriate to assign a maximum (least negative) slope in order to extrapolate a worst case limit value.

It was considered more appropriate to accumulate data so that the extrapolated values average can tend towards the true value. Due to the low level of the extrapolated values, the correlation between readings was considered sufficient for the purposes of notification.

The test material darkened slightly under the conditions used in the determination.

6.4 Conclusion

The vapour pressure of the test material has been determined to be 5.3×10^{-6} Pa at 25 °C.



Study Reference Number: KY030438

7. SURFACE TENSION

7.1 Method

The determination was carried out using a White Electrical Institute interfacial tension balance and a procedure based on the ISO 304 ring method. With the exception of the following deviation, the experimental procedure used complied with that specified in Method A5 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

- 1) The surface tension result was not corrected using the Harkins-Jordan correction table as the correction is not applicable to the apparatus used. Once calibrated, the balance and ring assembly used in this test give a direct reading for surface tension that is within the required accuracy (± 0.5 mN/m); this is as a result of the reduced ring dimensions.

This deviation has been considered not to have affected the integrity of the study.

7.1.1 Procedure

7.1.1.1 *Cleaning of apparatus*

The platinum ring and all glassware were cleaned with acetone, tap water, chromic acid, tap water and finally glass double-distilled water. The platinum ring was dried over a methanol flame and cleaned between each surface tension measurement.

7.1.1.2 *Preparation of sample solution*

Table 7.1

Sample Preparation	Time (mins)
An aliquot (0.1003 g) of test material was diluted to 100 ml with glass double-distilled water.	0 – 3
The sample solution was shaken by hand for 1 minute.	3 – 4
The sample solution was transferred to the measuring vessel.	4

[REDACTED] Study Reference Number: KY030438

7.1.1.3 Surface tension readings

The surface tension of the sample solution was measured at intervals until a constant reading was obtained. The elapsed time from transferral to the measuring vessel to obtaining each surface tension reading was recorded.

A calibration reading of glass double-distilled water was taken with each sample reading.

Readings were taken of the minimum force required to detach the ring from the surface of the liquid. Temperature readings were taken directly after each sample and calibration reading.

7.1.2 Calculation

7.1.2.1 Calibration factor (ϕ)

The calibration factor (ϕ), by which the measured surface tension of the sample solution is multiplied, was determined using Equation 7.1.

Equation 7.1

$$\phi = \frac{V_L}{V_M}$$

where:

- ϕ = calibration factor
- V_L = literature value for surface tension of water at test temperature (mN/m)
- V_M = measured value for surface tension of water at test temperature (mN/m)

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7.2 Results

7.2.1 Calibration factor (ϕ)

The readings, temperatures and the corresponding calibration factors for glass double-distilled water are shown in the following table:

Table 7.2

Reading (mN/m)	Temperature (°C)	Literature Value (mN/m)	Calibration Factor
72.5	19.4	72.84	1.005
73.0	19.4	72.84	0.998
72.5	19.6	72.81	1.004
72.5	19.6	72.81	1.004
72.5	19.6	72.81	1.004
72.5	19.8	72.78	1.004
72.5	19.8	72.78	1.004
Mean Calibration Factor (of last six readings) = 1.003			


7.2.2 Sample solution readings

The readings, times and temperatures for the sample solution are shown in the following table:

Table 7.3

Time (mins)	Reading (mN/m)	Temperature (°C)
67	70.0	19.4
79	70.5	19.6
86	70.5	19.6
93	70.5	19.6
101	70.5	19.8
113	70.5	19.8

Mean (of last five readings) = 70.5 mN/m
 Surface tension = reading x calibration factor
 = 70.5×1.003
 = 70.7 mN/m
 Temperature : $19.5 \pm 0.5^\circ\text{C}$
 pH of sample solution : 4.4

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7.3 Discussion

Substances having a surface tension below 60 mN/m are regarded as being surface-active.

Based on the pH of the sample solution and information obtained in the hydrolysis study (SPL Project Number 1736/023) it was considered that negligible hydrolysis of the sample solution occurred during the course of the surface tension test.

7.4 Conclusion

The surface tension of a 1.00 g/l solution of test material has been determined to be 70.7 mN/m at $19.5 \pm 0.5^\circ\text{C}$. The test material is considered not to be a surface-active material.

 Study Reference Number: KY030438

8. WATER SOLUBILITY

8.1 Method

The determination was carried out using the flask method, Method A6 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

8.1.1 Procedure

8.1.1.1 Preliminary test

An aliquot (3.0450g) of test material was diluted to 15 ml with glass double-distilled water. After shaking at 30°C for 5 hours and standing at 20°C for 17 hours, the solution was centrifuged at 13,500 rpm for approximately 30 minutes, filtered through 0.45 µm filter and analysed.

8.1.1.2 Definitive test

Based on the preliminary result, mixtures (see following table) of test material and glass double-distilled water were added to three separate flasks.

Table 8.1

Sample Number	Mass of Test Material (g)	Volume of Water (ml)
1	6.0129	15
2	6.0063	15
3	6.0094	15

After addition of glass double-distilled water to the flasks, they were shaken at approximately 30°C and, after standing at 20°C for a period of not less than 24 hours, the contents of the flasks were centrifuged at 13,500 rpm for approximately 30 minutes, sampled using syringes and needles, decanted and filtered through 0.45 filters. After this procedure, the samples were free from undissolved test material.

The pH of each solution was measured.

[REDACTED] Study Reference Number: KY030438

8.1.1.3 Analysis of sample solution

The concentration of test material in the sample solutions was determined by high performance liquid chromatography (HPLC).

Samples

Duplicate aliquots (A and B) of the sample solution were diluted by a factor of 1000 using acetonitrile.

Blank

Acetonitrile

Standards


Duplicate standard solutions of test material were prepared in acetonitrile at a nominal concentration of 75 mg/l.

Analysis

The standard and sample solutions were analysed by HPLC using the following conditions:

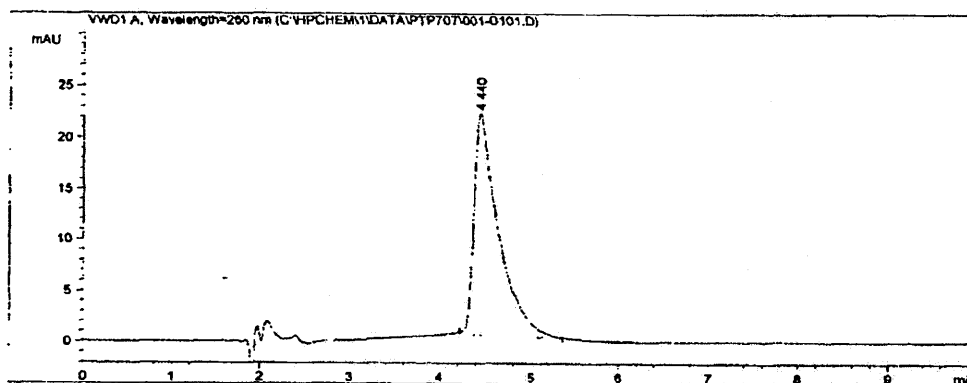
HPLC System	:	Agilent Technologies 1100, incorporating autosampler and workstation
Column	:	Spherisorb S5 C ₆ (250 x 4.6 mm id)
Column temperature	:	30°C
Mobile phase	:	buffer *: acetonitrile (65:35 v/v)
Flow-rate	:	1.5 ml/min
UV detector wavelength	:	260 nm
Injection volume	:	10 µl
Retention time	:	~ 4 mins

* buffer preparation – approximately 2.1628 g of 1-octane sulfonic acid sodium salt dissolved in 650 ml reverse osmosis water. Approximately 1.012 g of triethylamine added and the pH adjusted to 2.5 using orthophosphoric acid, or equivalent volumes/weights.

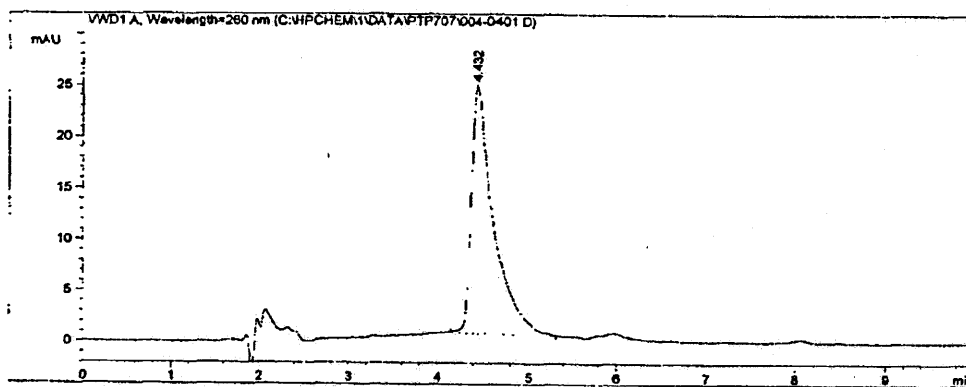
 Study Reference Number: KY030438

Typical Chromatography

Standard 75.5 mg/l



Sample 1A



[REDACTED] Study Reference Number: KY030438

8.1.2 Calculation

The mean peak area of each standard was corrected to a nominal concentration of 75 mg/l and the mean value taken.


The sample solution concentration (g/l) was calculated using Equation 8.1

Equation 8.1

$$C_{spl} = \frac{P_{spl}}{P_{std}} \times C_{std} \times D \times \frac{1}{1000}$$

where:

C_{spl}	=	sample concentration (g/l)
P_{spl}	=	mean peak area of sample solution
P_{std}	=	mean peak area of standard solution, corrected to nominal standard concentration
C_{std}	=	nominal standard concentration (75 mg/l)
D	=	sample dilution factor (1000)

 Study Reference Number: KY030438

8.2 Results

8.2.1 Preliminary test

The preliminary estimate of water solubility was 80.0 g/l.

8.2.2 Definitive test

The mean peak areas relating to the standard and sample solutions are shown in the following table:

Table 8.2

Solution	Mean Peak Area
Standard 75.5 mg/l	397.802
Standard 75.1 mg/l	398.099
Blank	ND*
Sample 1A	411.841
Sample 1B	405.259
Sample 2A	406.698
Sample 2B	410.012
Sample 3A	411.460
Sample 3B	414.929

The concentration (g/l) of test material in the sample solutions is shown in the following table:

Table 8.3

Sample Number	Time Shaken at ~ 30°C (hours)	Time Equilibrated at 20°C (hours)	Concentration (g/l)	Solution pH
1	24	24	77.3	2.7
2	48	24	77.2	2.7
3	72	24	78.1	2.8

Mean concentration: 77.5 g/l at $20.0 \pm 0.5^\circ\text{C}$

Range: 77.2 to 78.1 g/l

* ND = None detected.



Study Reference Number: KY030438

8.3 Validation

The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 0 to 160 mg/l. This was satisfactory with a correlation coefficient of 1.00 being obtained.

8.4 Discussion

It is evident from the information obtained in the hydrolysis study (see SPL Project number 1736/023) and data relating to the pH of the test material in water that negligible hydrolysis of the sample solution occurred during the course of the water solubility test.

8.5 Conclusion

The water solubility of the test material has been determined to be 77.5 g/l of solution at $20.0 \pm 0.5^{\circ}\text{C}$.

[REDACTED] Study Reference Number: KY030438

9. PARTITION COEFFICIENT

9.1 Method

The determination was carried out using the shake-flask method, Method A8 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

9.1.1 Procedure

9.1.1.1 Reagents

Mutually saturated n-octanol and water was prepared in-house.

9.1.1.2 Preliminary estimation

A preliminary assessment of the partition coefficient was made based on the approximate solubilities of the test material in n-octanol and water.

9.1.1.3 Definitive test

A stock solution was prepared by diluting test material (0.5078 g) to 250 ml with n-octanol saturated water. The pH was adjusted to 7.5 using 0.1M sodium hydroxide.

Six partitions (see following table) were performed. In each test, the combined volume of both phases occupied not less than 90% of the total volume of the test vessel.

Table 9.1

Test	Volume of Stock Solution (ml)	Volume of Water Saturated n-octanol (ml)	Temperature (°C)
1	40	80	19.8
2	40	80	20.0
3	25	100	20.0
4	25	100	20.0
5	15	120	21.6
6	15	120	20.2

[REDACTED] Study Reference Number: KY030438

The shaking was performed by inversion of the flasks through approximately 180° over a five minute period. After separation, aliquots of both phases were taken for analysis.

9.1.1.4 Analysis of sample solutions

The concentration of test material in the sample solutions was determined by high performance liquid chromatography (HPLC).

Organic phase samples

The samples were diluted by a factor of 2 using acetonitrile.

Organic phase standards

Duplicate standard solutions of test material were prepared in acetonitrile:water saturated n-octanol (50:50 v/v) at a nominal concentration of 10 mg/l.

Organic phase blank

Acetonitrile:water saturated n-octanol (50:50 v/v).

Aqueous phase samples

The samples were diluted by a factor of 20 using acetonitrile.

Duplicate aliquots (A and B) of the stock solution were diluted by a factor of 20 using acetonitrile.

Aqueous phase standards

Duplicate standard solutions of test material were prepared in acetonitrile:n-octanol saturated water (95:5 v/v) at a nominal concentration of 100 mg/l.

Aqueous phase blank

Acetonitrile:n-octanol saturated water (95:5 v/v).




Study Reference Number: KY030438

Analysis

The standard and sample solutions were analysed by HPLC using the following conditions:

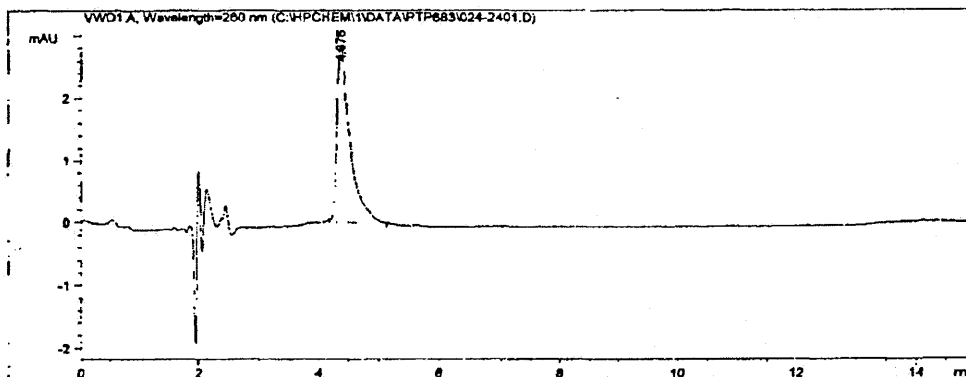
HPLC System	:	Agilent Technologies 1100, incorporating autosampler and workstation
Column	:	Spherisorb S5 C ₆ (250 x 4.6 mm id)
Column temperature	:	30°C
Mobile phase	:	buffer *:acetonitrile (65:35 v/v)
Flow-rate	:	1.5 ml/min
UV detector wavelength	:	260 nm
Injection volume	:	10 µl
Retention time	:	~ 4 mins

* buffer preparation – approximately 2.1628 g of 1-octane sulfonic acid sodium salt dissolved in 650 ml reverse osmosis water. Approximately 1.012 g of triethylamine added and the pH adjusted to 2.5 using orthophosphoric acid, or equivalent volumes/weights.

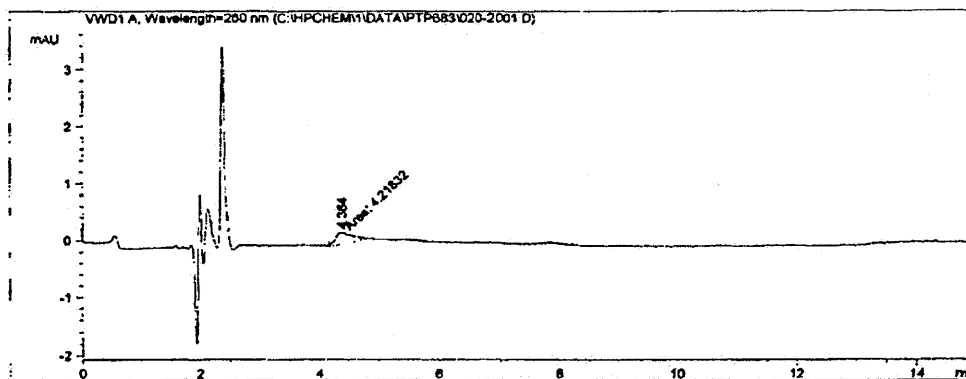
 Study Reference Number: KY030438

Typical Chromatography

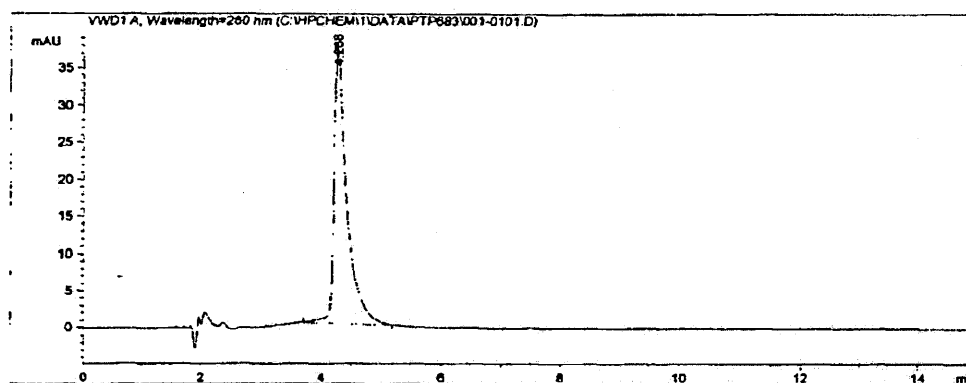
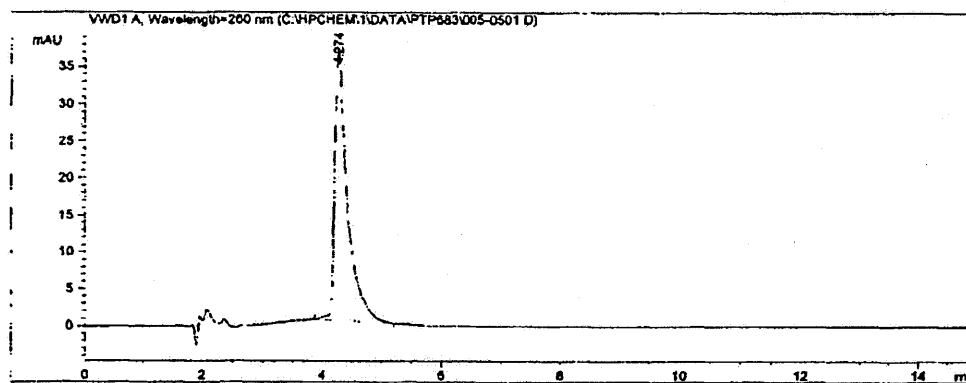
Organic Phase - Standard 10.3 mg/l



Organic Phase -- Sample 4



[REDACTED] Study Reference Number: KY030438

Typical Chromatography**Aqueous Phase - Standard 102 mg/l****Aqueous Phase - Sample 1**

[REDACTED] Study Reference Number: KY030438

9.1.2 Calculation

9.1.2.1 Preliminary estimate

The preliminary estimate of the partition coefficient was calculated using Equation 9.1.

Equation 9.1

$$P_{ow} \text{ estimate} = \frac{\text{solubility of the test material in n - octanol}}{\text{solubility of the test material in water}}$$

9.1.2.2 Definitive test

The mean peak area of each standard was corrected to nominal concentration and the mean value taken.

The analysed concentrations (mg/l) of the organic, aqueous and stock solutions were calculated using Equation 9.2, Equation 9.3 and Equation 9.4 respectively.

Equation 9.2

$$C_{org} = \frac{sple_{org}}{std_{org}} \times C_{std} \times D$$

Equation 9.3

$$C_{aq} = \frac{sple_{aq}}{std_{aq}} \times C_{std} \times D$$

Equation 9.4

$$C_{stock} = \frac{stock}{std_{org}} \times C_{std} \times D$$

The total weights (mg) of test material in the respective phases were calculated using Equation 9.5, Equation 9.6 and Equation 9.7 respectively.

Equation 9.5

$$W_{org} = C_{org} \times \frac{V_{org}}{1000}$$

[REDACTED] Study Reference Number: KY030438

Equation 9.6

$$W_{aq} = C_{aq} \times \frac{V_{aq}}{1000}$$

Equation 9.7

$$W_{stock} = C_{stock} \times \frac{V_{stock}}{1000}$$

The partition coefficient for each determination was calculated using Equation 9.8.

Equation 9.8

$$P_{ow} = \frac{C_{org}}{C_{aq}}$$

where:

C_{stock}	=	analysed stock solution concentration (mg/l)
C_{org}	=	analysed organic phase concentration (mg/l)
C_{aq}	=	analysed aqueous phase concentration (mg/l)
stock	=	mean peak area for stock solution
sple _{org}	=	mean peak area for organic phase solution
sple _{aq}	=	mean peak area for aqueous phase solution
std _{org}	=	mean peak area for organic standard solution, corrected to nominal standard concentration
std _{aq}	=	mean peak area for aqueous standard solution, corrected to nominal standard concentration
C_{std}	=	nominal standard concentration (mg/l)
D	=	dilution factor
W_{stock}	=	weight of test material found in the stock solution (mg)
W_{org}	=	weight of test material found in the organic phase (mg)
W_{aq}	=	weight of test material found in the aqueous phase (mg)
V_{stock}	=	volume of stock solution used in the determination (ml)
V_{org}	=	volume of organic phase used in the determination (ml)
V_{aq}	=	volume of aqueous phase used in the determination (ml)
P_{ow}	=	partition coefficient

 Study Reference Number: KY030438

9.2 Results

9.2.1 Preliminary estimate

Approximate solubility in n-octanol: <16.0 mg/l

Approximate solubility in water: 1.39×10^5 mg/l

Approximate P_{ow} : $<1.15 \times 10^{-4}$

$\text{Log}_{10} P_{ow}$: <-3.94

9.2.2 Definitive test

The mean peak areas obtained for the standard, stock and sample solutions are shown in the following two tables:

Table 9.2 – Aqueous Phase

Solution	Mean Peak Area
Standard 102 mg/l	531.765
Standard 103 mg/l	545.256
Blank	ND*
Sample 1	536.883
Sample 2	533.953
Sample 3	536.938
Sample 4	509.412
Sample 5	519.745
Sample 6	477.246
Stock solution A	507.215
Stock solution B	526.043

* ND = None detected.



Study Reference Number: KY030438

Table 9.3 – Organic Phase

Solution	Mean Peak Area
Standard 10.2 mg/l	47.622
Standard 10.3 mg/l	44.908
Blank	ND*
Sample 1	4.004
Sample 2	3.814
Sample 3	4.175
Sample 4	4.873
Sample 5	3.743
Sample 6	3.692

The total weights (mg) and analysed concentration (mg/l) of the respective phases are shown in the following table:

Table 9.4

Sample Number	Volume of Water Saturated n-octanol (ml)	Volume of n-octanol Saturated Water (ml) Stock Solution (ml)	Total Weight (mg)*	Organic Phase		Aqueous Phase		
				Analysed Concentration (mg/l)	Total Weight (mg)†	Analysed Concentration (mg/l)	Total Weight (mg)†	pH
1	80	40	78.8	1.78	0.142	2.05×10^3	81.9	6.5
2	80	40	78.8	1.69	0.135	2.04×10^3	81.4	6.5
3	100	25	49.2	1.85	0.185	2.05×10^3	51.2	6.6
4	100	25	49.2	2.16	0.216	1.94×10^3	48.6	6.7
5	120	15	29.5	1.66	0.199	1.98×10^3	29.7	6.7
6	120	15	29.5	1.64	0.197	1.82×10^3	27.3	6.6

pH of n-octanol saturated water: 5.8

pH of stock solution: 7.5

Temperature: $20.6 \pm 1^\circ\text{C}$

* From analysis of the stock solution

† From analysis of the respective phase

[REDACTED] Study Reference Number: KY030438

The partition coefficient determined for each sample is shown in the following table:

Table 9.5

Sample Number	n-Octanol/Water Volume Ratio	Partition Coefficient	Log ₁₀ P _{ow}	Mean Partition Coefficient
1	2:1	8.68×10^{-4}	-3.06	8.50×10^{-4}
2		8.31×10^{-4}	-3.08	
3	4:1	9.05×10^{-4}	-3.04	1.01×10^{-3}
4		1.11×10^{-3}	-2.95	
5	8:1	8.38×10^{-4}	-3.08	8.69×10^{-4}
6		9.00×10^{-4}	-3.05	

Mean P_{ow} : 9.09×10^{-4}

log₁₀ P_{ow} :-3.04

Standard deviation : 1.05×10^{-4}

9.3 Validation

The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 0 to 200 mg/l for the aqueous sample matrix and 0 to 20 mg/l for the organic sample matrix. Both linearities were satisfactory with correlation coefficients of 0.997 and 0.998 respectively being obtained.

9.4 Discussion

Substances having a log₁₀ P_{ow} of less than 3 are regarded as not having the potential to bioaccumulate in the environment.

The n-octanol saturated water was adjusted to pH 7.5 to ensure the test material was partitioned at an environmentally relevant pH.

9.5 Conclusion

The partition coefficient of the test material has been determined to be 9.09×10^{-4} at $20.6 \pm 1.0^\circ\text{C}$, log₁₀ P_{ow} -3.04.



Study Reference Number: KY030438

10. FLAMMABILITY (SOLIDS)

10.1 Method

The flammability (solids) was determined by measuring the burning rate of test material prepared as a pile of set dimensions. Testing was conducted using Method A10 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

10.1.1 Procedures

10.1.1.1 Preliminary screening test

A mould (250 x 20 x 10 mm) was loosely filled with test material (tested as received). A non-combustible, non-porous board was placed onto the mould which was then inverted. The mould was removed and an air-rich Bunsen burner flame applied to one end of the pile until ignition occurred. The time taken to propagate 200 mm was recorded.

10.1.1.2 Moisture content

The moisture content was determined gravimetrically.

An aliquot (approximately 1 g) of test material was weighed (in duplicate; A and B) into loss bottles. The samples were dried to constant weight at approximately 105°C.

10.1.2 Calculation


The moisture content was calculated using Equation 10.1.

Equation 10.1

$$\text{Moisture Content (\%)} = \frac{b - c}{b - a} \times 100$$

Where:

- a = mass of loss bottle (g)
- b = mass of loss bottle and test material (g)
- c = mass of loss bottle and test material after drying (g)

 Study Reference Number: KY030438

10.2 Results

10.2.1 Preliminary screening test

The pile did not ignite, although it smouldered across 200 mm in 1 hour 43 minutes and 29 seconds.

The result of the preliminary screening test obviated the need to perform the main test.

10.2.2 Moisture content

The results of the moisture content are shown in the following table:

	Determination A	Determination B
a) Mass of loss bottle (g)	18.56070	19.48467
b) Mass of loss bottle and test material (g)	19.56247	20.50073
c) Mass of loss bottle and test material after drying (g)	19.53360	20.47072
Moisture content (% w/w)	2.882	2.954
Mean moisture content (% w/w)	2.918	

10.3 Conclusion

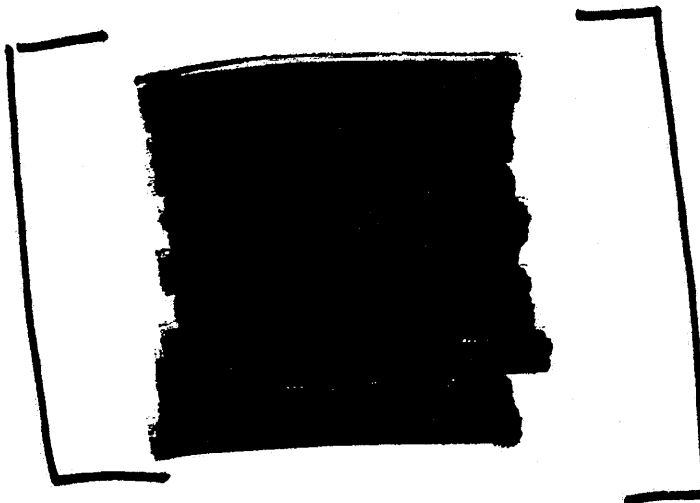
The test material has been determined to be not highly flammable as it did not propagate combustion over the 200 mm of the preliminary screening test in under 4 minutes.

 Study Reference Number: KY030438

11. EXPLOSIVE PROPERTIES

11.1 Summary

Based on the chemical structure of the test material it was considered unnecessary to carry out the explosive properties test according to Method A14 of Commission Directive 92/69/EEC. There are no significant functional groups that infer explosive properties. Therefore, the test result has been predicted as negative.



[REDACTED] Study Reference Number: KY030438

12. RELATIVE SELF-IGNITION TEMPERATURE FOR SOLIDS

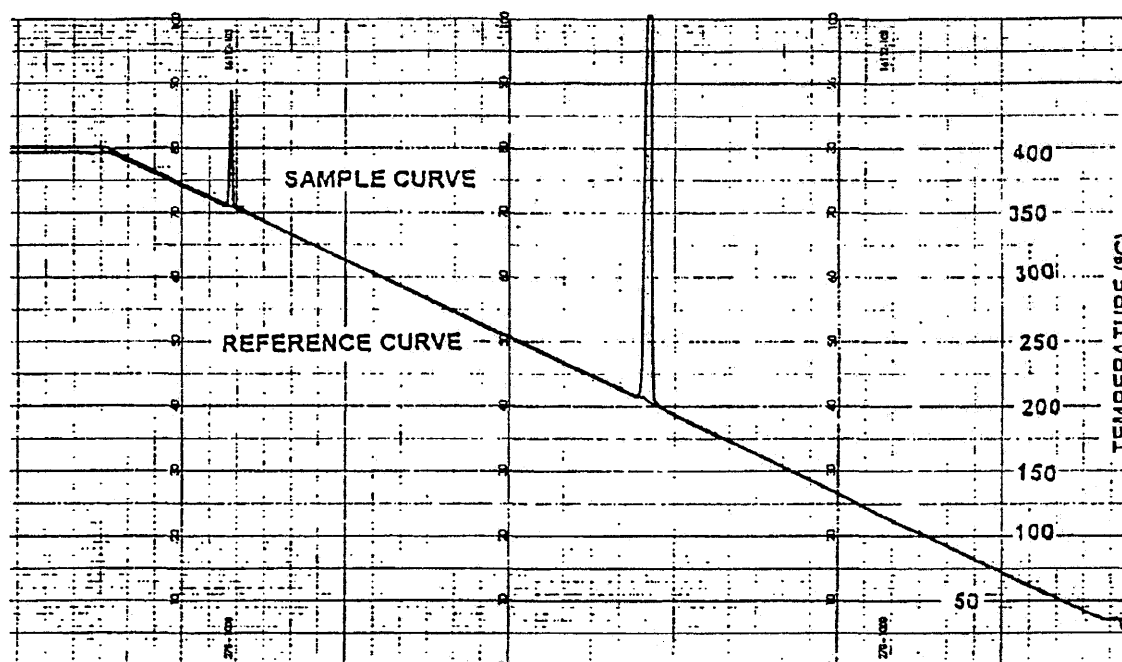
12.1 Method

The test material was heated in an oven and the relative self ignition temperature determined. Testing was conducted using Method A16 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

12.1.1 Procedure

An aliquot of the test material was suspended in a stainless steel mesh cube (approximately 20 x 20 x 20 mm) in an oven. A thermocouple was placed in the centre of the sample and another in the oven. The oven temperature was programmed to increase from ambient to 400°C at a rate of 0.5°C/min. The temperature/time curves relating to the condition in the centre of the sample and the oven were recorded on a two channel chart recorder.

12.2 Results



[REDACTED] Study Reference Number: KY030438

Observations after the test

The cube contained [REDACTED] charred material.

12.3 Conclusion

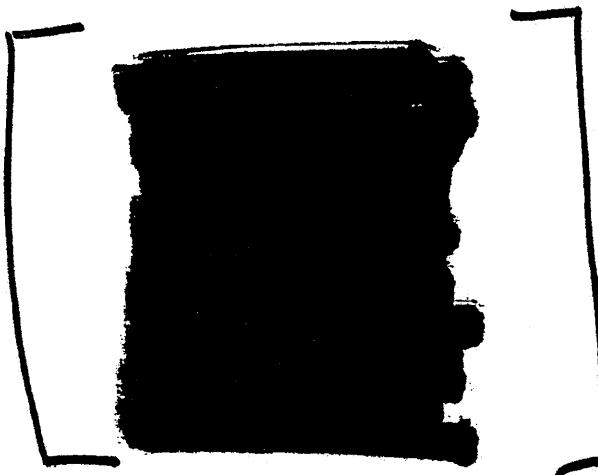
The test material has been determined to have a relative self-ignition temperature of 202°C.

[REDACTED] Study Reference Number: KY030438

13. OXIDISING PROPERTIES

13.1 Summary

Based on the chemical structure of the test material it was considered unnecessary to carry out the oxidising properties according to Method A17 of Commission Directive 92/69/EEC. There are no significant functional groups that infer oxidising properties. Therefore, the test result has been predicted as negative.



[REDACTED] Study Reference Number: KY030438

Appendix 1 Statement of GLP Compliance in accordance with Directive 88/320/EEC**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

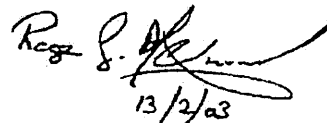
LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION**2nd December 2002**

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above Laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


13/2/03

Dr Roger G. Alexander
Head, UK GLP Monitoring Authority

**Study Reference Number: KY030438**

RCC Study Number 851817

[REDACTED] Study Reference Number KO030437

[REDACTED]

Acute Oral Toxicity Study in Rats

Report

Author: G. Arcelin

Sponsor: [REDACTED]



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RCC STUDY NUMBER 851817
Study Reference Number KO030437

Report

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1 PREFACE

1.1 GENERAL

Title

Acute Oral Toxicity Study in Rats

Sponsor

Project Planing
Contact NamesMrs L. Selbie
Mrs C. Talbot
Mrs T. Gübler
Miss J. Evans

Scientific Representative

Miss K. Wilson

Test Facility

RCC Ltd
Toxicology
Operational Unit: Safety Assessment I
Wölferstrasse 4
CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director

G. Arcelin

Technical Coordinator

P. Reissbrodt

Head of RCC Quality
Assurance

I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date 05-DEC-2003

Experimental Completion Date 05-FEB-2004

Delivery of Animals

05-DEC-2003 (females, 300 mg/kg)
09-DEC-2003 (females, 300 mg/kg)
17-DEC-2003 (females, 50 mg/kg)
19-DEC-2003 (females, 50 mg/kg)
13-JAN-2004 (females, 200 mg/kg)
15-JAN-2004 (females, 200 mg/kg)

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Study Reference Number KO030437

Acclimatization	05-DEC-2003 to 11-DEC-2003 (females, 300 mg/kg) 09-DEC-2003 to 15-DEC-2003 (females, 300 mg/kg) 17-DEC-2003 to 21-DEC-2003 (females, 50 mg/kg) 19-DEC-2003 to 23-DEC-2003 (females, 50 mg/kg) 13-JAN-2004 to 19-JAN-2004 (females, 200 mg/kg) 15-JAN-2004 to 21-JAN-2004 (females, 200 mg/kg)
Treatment	12-DEC-2003 (females, 300 mg/kg) 16-DEC-2003 (females, 300 mg/kg) 22-DEC-2003 (females, 50 mg/kg) 24-DEC-2003 (females, 50 mg/kg) 20-JAN-2004 (females, 200 mg/kg) 22-JAN-2004 (females, 200 mg/kg)
Observation	05-DEC-2003 to 26-DEC-2003 (females, 300 mg/kg) 09-DEC-2003 to 16-DEC-2003 (females, 300 mg/kg) 17-DEC-2003 to 05-JAN-2004 (females, 50 mg/kg) 19-DEC-2003 to 07-JAN-2004 (females, 50 mg/kg) 13-JAN-2004 to 03-FEB-2004 (females, 200 mg/kg) 15-JAN-2004 to 05-FEB-2004 (females, 200 mg/kg)
Study Completion Date	17-MAR-2004

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, amendment, raw data, a sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

The remaining test item will be returned to the Sponsor. It is the Sponsor's responsibility to archive the test item.

RCC STUDY NUMBER 851817

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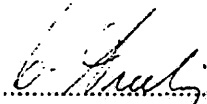
Page 5

[REDACTED] Study Reference Number KO030437

1.5 SIGNATURE PAGE

Study Director:

G. Arcelin


.....
date: 17. MAR. 2004

Management:

(for) Dr. H. Fankhauser


.....
date: 16. MAR. 2004

RCC STUDY NUMBER 851817
Study Reference Number KO030437

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 851817
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Acute Oral Toxicity Study in Rats

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures, with the exception of the trial formulation, were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
04-DEC-2003 Study Plan	04-DEC-2003
16-DEC-2003 Test System, Raw Data, Necropsy	16-DEC-2003
08-MAR-2004 Report	08-MAR-2004

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

M. C. Schlepper

M. C. Schlepper
date: 17. März - 2004

RCC STUDY NUMBER 851817

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Study Reference Number KO030437

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER : 851817

TEST ITEM :

STUDY DIRECTOR : G. Arcelin

TITLE :

Acute Oral Toxicity Study in Rats

The supporting data for purity (characterisation), stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the Statement of Compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449. The solubility trials, to determine the choice of vehicle, were performed before the study initiation date and therefore are also excluded from this Statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director:

G. Arcelin

date: 17 MAR 2004

BCC STUDY NUMBER 851817

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Study Reference Number KO030437

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17th December 2001.

Directive 96/54/EEC, B.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", September 30, 1996.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 34.

1.10 SUMMARY OF STUDY PLAN AMENDMENT

Study Plan Amendment No. 1:

A further dose level of 200 mg/kg was required by the sponsor in order to adapt the test to EU regulatory classifications, Annex 5.

RCC STUDY NUMBER 851817

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Study Reference Number KO030437

2 SUMMARY

Six groups, each of three female HanBrl: WIST (SPF) rats, were treated with [REDACTED] by oral gavage administration at dosages of 300 mg/kg, 200 mg/kg or 50 mg/kg body weight. The test item was diluted in vehicle (purified water) at concentrations of 0.03 g/mL, 0.02 g/mL or 0.005 g/mL and administered at a volume dosage of 10 mL/kg.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs at approximately 0 (300 and 200 mg/kg dose groups only), 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded twice daily during test days 1-15. Body weights were recorded on day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

The following animals were treated and percentage of mortality was observed:

6 females treated at 300 mg/kg	67 %
6 females treated at 200 mg/kg	17 %
6 females treated at 50 mg/kg	0 %

Three 300 mg/kg treated animals were found dead approximately 15 to 20 minutes after test item administration and one animal of this dose group had to be killed in extremis for ethical reasons immediately after the 2-hour reading. One animal treated at 200 mg/kg was found dead at the 1-hour reading. All 50 mg/kg treated animals and the remaining animals of the other two dose groups survived until the end of the study period.

Sedation, ventral recumbency and slight to moderate convulsions were observed in five 300 mg/kg treated animals at the 0-hour reading and, except sedation, persisted in one animal up to the 1-hour reading. Lateral recumbency was noted in one animal and bradypnea in three animals at the 0-hour reading before death occurred. One animal of this dose group was in a moribund state at the 2-hour reading before it was killed.

Slight ataxia was observed in two 200 mg/kg treated animals from the 0- or 1- to the 2-hour reading. The same two animals showed slightly ruffled fur from the 1- to the 5-hour reading and hunched posture from the 2- to the 3-hour reading. Sedation was noted in two animals at the 0-hour reading and also at the 1-hour reading for one of the animals. Ventral recumbency and slight convulsions were also observed at the 0-hour reading in two animals. One animal of this dose group was seen with bradypnea at the 0-hour reading before being found dead at the 1-hour reading.

No clinical signs were observed in three 200 mg/kg in all 50 mg/kg treated animals during the course of the study.

The body weight of the animals was within the range commonly recorded for this strain and age.

One 300 mg/kg treated animal was observed with a heart reduced in size and one animal treated at 200 mg/kg was noted with liquid contents in its stomach. No macroscopic findings were recorded in the remaining animals of these two dose groups and in all 50 mg/kg treated animals at scheduled and unscheduled necropsies.

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Study Reference Number KO030437

It should be noted that, contrary to the method outlined in the protocol, the three animals in the second 300 mg/kg dose group were not fasted prior to treatment. This error does not appear to have influenced the sensitivity of the study because all three of these animals died, or were killed in extremis, soon after treatment, therefore the conclusion (median lethal dose of [REDACTED]) is not affected.

3 CONCLUSION

The median lethal dose of [REDACTED] after single oral administration to female rats, observed over a period of 14 days is:

Oral LD₅₀ (rat) > 200 mg/kg body weight < 300 mg/kg body weight

RCC STUDY NUMBER 851817
Study Reference Number KO030437

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4 PURPOSE

The purpose of this study was to assess the acute toxicity of [REDACTED] when administered by a single oral gavage to rats, followed by an observation period of 14 days.

This study provides information for both hazard assessment and hazard classification purposes.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Rat, HanBrl: Wist (SPF)
Rationale	Recognized by the international guidelines as a recommended test system.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals per group	3 females
Total number of animals	18 females
Age when treated	8 - 10 weeks
Identification	Unique cage number and corresponding color-coded spots on the tail. The animals were marked at acclimatization start.
Randomization	Selected by hand at time of delivery. No computer generated randomization program.
Acclimatization	Under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

5.2 HUSBANDRY

Room no.	104 / RCC Ltd, Füllinsdorf
Conditions	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with target ranges for room temperature 22 ± 3 °C and for relative humidity between 30-70 % (values above 70 % during cleaning process possible), automatically controlled light cycle of 12 hours light and 12 hours dark, music during the daytime light period.
Accommodation	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 Muttensz/Switzerland).

RCC STUDY NUMBER 851817

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Study Reference Number KO030437

Diet Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch nos. 54/03 and 78/03 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland) *ad libitum*. Results of analyses for contaminants are archived at RCC Ltd, Itingen.

Water Community tap water from Füllinsdorf *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

5.3 TEST ITEM

The following information was provided by the sponsor:

Identification

Description

Sample number

S2539801

Purity

The supporting data for purity of the test item was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study, Study Reference Number AC030449.

Expiry date

01-JAN-2005

Stability of test item dilution

Stability of the dosing solutions was addressed by ensuring fresh solutions were made immediately prior to dosing.

Storage conditions

At room temperature (range of 20 ± 3 °C), protected from light.

Safety precautions

Routine hygienic procedures were used to ensure the health and safety of the personnel.

The supporting data for stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study, Study Reference Number AC030449.

5.4 VEHICLE

A solubility trial was carried out to determine the choice of vehicle. This was a non-GLP trial, performed before the study initiation date, and therefore is excluded from the statement of compliance.

RCC STUDY NUMBER 851817
Study Reference Number KO030437

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The following information was provided by RCC Ltd:

Purified water prepared at RCC Ltd (deionised water which was processed and treated by the PURELAB Option-R unit. This latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).

Purified water was found to be a suitable vehicle.

5.5 DOSE FORMULATION

Dose levels are in terms of the test item as supplied by the sponsor.

The dose formulations were made shortly before each dosing occasion using a magnetic stirrer and spatula as homogenizers.

The test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added (weight:volume).

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

5.6 TREATMENT

The animals received a single dose of the test item by oral gavage administration at 300 mg/kg, 200 mg/kg or 50 mg/kg body weight after being fasted for approximately 17 to 18 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. According to the raw data, no fasting period was respected in the three animals of the second 300 mg/kg treated group. The implications of this are discussed in the Summary (Section 2).

The application volume was 10 mL/kg body weight.

Rationale: Oral administration was considered to be an appropriate application method as it is a possible route of human exposure during manufacture, handling and use of the test item.

5.7 OBSERVATIONS

Mortality / Viability	Daily during acclimatization and twice daily during days 1-15.
Body weights	On test days 1 (prior to administration), 8 and 15.
Clinical signs	Daily during acclimatization and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.

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Study Reference Number KO030437

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6 PATHOLOGY

6.1 NECROPSY

All animals which died spontaneously or were killed in extremis for ethical reasons during the observation period were necropsied as soon as they were found dead or killed.

All surviving animals were killed at the end of the observation period by an intraperitoneal injection of Vetanarcol at a dose of at least 2.0 mL/kg body weight (equivalent to at least 324 mg sodium pentobarbitone/kg body weight) and discarded after macroscopic examinations were performed. No organs or tissues were retained.

7 STATISTICAL ANALYSIS

No statistical analysis was used.

8 DATA COMPILATION

Body weights were recorded on-line.

Clinical signs were recorded on data sheets.

Mortality/viability were compiled into the RCC Tox Computer System during recording and/or recorded on data sheets.

Macroscopic findings were compiled into the RCC Tox Computer System during recording or recorded on data sheets.

The RCC Tox Computer System (RCC-Tox-Lims) had been validated with respect to data collection, storage and retrievability.

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Study Reference Number KO030437

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9 RESULTS

9.1 MORTALITY

Three 300 mg/kg treated animals were found dead approximately 15 to 20 minutes after test item administration and one animal of this dose group had to be killed in extremis for ethical reasons immediately after the 2-hour reading. One animal treated at 200 mg/kg was found dead at the 1-hour reading. All 50 mg/kg treated animals and the remaining animals of the other two dose groups survived until the end of the study period.

9.2 CLINICAL SIGNS

Sedation, ventral recumbency and slight to moderate convulsions were observed in five 300 mg/kg treated animals at the 0-hour reading and, except sedation, persisted in one animal up to the 1-hour reading. Lateral recumbency was noted in one animal and bradypnea in three animals at the 0-hour reading before death occurred. One animal of this dose group was in a moribund state at the 2-hour reading before it was killed.

Slight ataxia was observed in two 200 mg/kg treated animals from the 0- or 1- to the 2-hour reading. The same two animals showed slightly ruffled fur from the 1- to the 5-hour reading and hunched posture from the 2- to the 3-hour reading. Sedation was noted in two animals at the 0-hour reading and also at the 1-hour reading for one of the animals. Ventral recumbency and slight convulsions were also observed at the 0-hour reading in two animals. One animal of this dose group was seen with bradypnea at the 0-hour reading before being found dead at the 1-hour reading.

No clinical signs were observed in three 200 mg/kg in all 50 mg/kg treated animals during the course of the study.

9.3 BODY WEIGHTS

The body weight of the animals was within the range commonly recorded for this strain and age.

9.4 MACROSCOPIC FINDINGS

One 300 mg/kg treated animal was observed with a heart reduced in size and one animal treated at 200 mg/kg was noted with liquid contents in its stomach. No macroscopic findings were recorded in the remaining animals of these two dose groups and in all 50 mg/kg treated animals at scheduled and unscheduled necropsies.

9.5 MEDIAN LETHAL DOSE

The median lethal dose of [REDACTED] after single oral administration to female rats, observed over a period of 14 days is:

Oral LD₅₀ (rat) > 200 mg/kg body weight < 300 mg/kg body weight

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 Study Reference Number KO030437

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10 INDIVIDUAL FINDINGS

10.1 MORTALITY / CLINICAL SIGNS

Dose mg/kg	Animal No.	Sex	Signs	Test days					2	3	4	5	6	7	8	9	10	11	12	13	14	15	
				1																			
				0*	1*	2*	3*	5*															
300	1	F	Lateral recumbency	√																			
			Convulsions	2																			
			Bradypnea	√																			
			Sedation	1+																			
	2	F	No clinical signs		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
			Ventral recumbency	√																			
			Sedation	1																			
	3	F	No clinical signs		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
			Ventral recumbency	√																			
			Convulsions	1																			
			Sedation	1																			
	300	4	F	Ventral recumbency	√																		
Convulsions				1																			
Bradypnea				√																			
Sedation				1+																			
5		F	Ventral recumbency	√																			
			Convulsions	1																			
			Bradypnea	√																			
			Sedation	1+																			
6		F	Ventral recumbency	√	√																		
			Convulsions	1	1																		
			Moribund			√ ^K																	

Key: 1 slight, 2 moderate, 3 marked, + found dead, ^K killed in extremis, √ noted

* Examinations were performed approximately 0, 1, 2, 3 and 5 hours after treatment.

Note: The animals nos. 4-5 were not fasted unlike the other groups.

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 Study Reference Number KO030437

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10.1 MORTALITY / CLINICAL SIGNS (CONTINUED)

Dose mg/kg	Animal No.	Sex	Signs	Test days					2	3	4	5	6	7	8	9	10	11	12	13	14	15	
				1																			
				0*	1*	2*	3*	5*															
50	7	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	8	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	9	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
50	10	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	11	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	12	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
200	13	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	14	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	15	F	No clinical signs	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
200	16	F	No clinical signs						√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
			Ataxia	1	1	1																	
			Ruffled fur		1	1	1	1															
			Hunched posture			√	√																
	17	F	No clinical signs						√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
			Sedation	1	1																		
			Ventral recumbency	√																			
			Convulsions	1																			
			Ataxia		1	1																	
			Ruffled fur		1	1	1	1															
			Hunched posture			√	√																
	18	F	Sedation	1																			
			Ventral recumbency	√																			
			Convulsions	1																			
			Bradypnea	√	+																		

Key: 1 slight, + found dead, √ noted, - no observation performed

* Examinations were performed approximately 0 (200 mg/kg dose groups only), 1, 2, 3 and 5 hours after treatment.

No clinical signs were evident in any animal during the acclimatization period.

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10.2 BODY WEIGHTS

Dose mg/kg	Animal No.	Sex	Day 1 (treatment)	Day 8	Day 15
300	1	F	157.3	-	-
	2	F	157.7	176.1	179.5
	3	F	161.9	182.6	191.9
300	4	F	159.3	-	-
	5	F	169.8	-	-
	6	F	170.1	-	-
50	7	F	166.5	195.0	207.8
	8	F	157.6	177.5	189.1
	9	F	177.0	202.4	213.5
50	10	F	161.9	188.7	199.3
	11	F	160.3	188.1	195.7
	12	F	161.3	182.7	191.9
200	13	F	165.8	184.2	193.1
	14	F	160.9	179.4	188.2
	15	F	159.1	182.4	190.1
200	16	F	168.4	196.9	213.3
	17	F	161.5	183.0	196.7
	18	F	176.8	-	-

Body weights are presented in grams.

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Study Reference Number KO030437

10.3 MACROSCOPIC FINDINGS

Dose mg/kg	Animal No.	Sex	Mode of death	Findings
300	1	F	D	Heart reduced in size
	2	F	S	No macroscopic findings
	3	F	S	No macroscopic findings
300	4	F	D	No macroscopic findings
	5	F	D	No macroscopic findings
	6	F	K	No macroscopic findings
50	7	F	S	No macroscopic findings
	8	F	S	No macroscopic findings
	9	F	S	No macroscopic findings
50	10	F	S	No macroscopic findings
	11	F	S	No macroscopic findings
	12	F	S	No macroscopic findings
200	13	F	S	No macroscopic findings
	14	F	S	No macroscopic findings
	15	F	S	No macroscopic findings
200	16	F	S	No macroscopic findings
	17	F	S	No macroscopic findings
	18	F	D	Liquid contents in the stomach

S: scheduled necropsy, D: found dead, K: killed in extremis

BCC STUDY NUMBER 851817
 Study Reference Number KO030437

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11 GLP – CERTIFICATION

The Swiss GLP Monitoring Authorities



Swiss Federal
Office of
Public Health



Swiss Agency for the
Environment, Forests
and Landscape

swissmedic

Swissmedic
Swiss Agency for
Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of

November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities

Areas of expertise *

- Toxicology

TOX, ACC, MUT,
OTH (Safety Pharmacology)

- Environmental Chemistry and
Pharmanalytics

ACC, ECT, ENF, EMN, PCT,
RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice (RS 813.016.5) at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology; ACC = Analytical and Clinical Chemistry; ECT = Environmental toxicity on aquatic and terrestrial organisms; ENF = Behaviour in water, soil and air, Bioaccumulation; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing; RES = Residue studies; OTH = Other, to be specified.

RCC Study Number 851818

[REDACTED] Study Reference Number KK030436

[REDACTED]

Acute Dermal Toxicity Study in Rats

Report

Author: G. Arcelin

Sponsor: [REDACTED]

BCC STUDY NUMBER 851818
Study Reference Number KK030436

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BCC STUDY NUMBER 851818

Report

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[REDACTED] Study Reference Number KK030436

1 PREFACE

1.1 GENERAL

Title

[REDACTED]
Acute Dermal Toxicity Study in Rats

Sponsor

[REDACTED] Project Planning
Contact Names

[REDACTED]
Mrs L. Selbie
Mrs C. Talbot
Mrs T. Gübler
Miss J. Evans

Scientific Representative

Miss K. Wilson

Test Facility

RCC Ltd
Toxicology
Operational Unit: Safety Assessment I
Wölferstrasse 4
CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director

G. Arcelin

Technical Coordinator

P. Reissbrodt

Head of RCC Quality
Assurance

I. Wüthrich

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Study Reference Number KK030436

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1.3 SCHEDULE

Experimental Starting Date	09-DEC-2003 (5 males and 5 females, 800 mg/kg) 03-MAR-2004 (1 female, 2000 mg/kg) 05-MAR-2004 (4 females, 2000 mg/kg) 09-MAR-2004 (5 males, 2000 mg/kg)
Experimental Completion Date	30-DEC-2003 (800 mg/kg) 24-MAR-2004 (1 female, 2000 mg/kg) 26-MAR-2004 (4 females, 2000 mg/kg) 30-MAR-2004 (5 males, 2000 mg/kg)
Delivery of Animals	09-DEC-2003 (800 mg/kg) 03-MAR-2004 (1 female, 2000 mg/kg) 05-MAR-2004 (4 females, 2000 mg/kg) 09-MAR-2004 (5 males, 2000 mg/kg)
Acclimatization	09-DEC-2003 to 15-DEC-2003 (800 mg/kg) 03-MAR-2004 to 09-MAR-2004 (1 female, 2000 mg/kg) 05-MAR-2004 to 11-MAR-2004 (4 females, 2000 mg/kg) 09-MAR-2004 to 15-MAR-2004 (5 males, 2000 mg/kg)
Treatment	16-DEC-2003 (800 mg/kg) 10-MAR-2004 (1 female, 2000 mg/kg) 12-MAR-2004 (4 females, 2000 mg/kg) 16-MAR-2004 (5 males, 2000 mg/kg)
Observation	09-DEC-2003 to 30-DEC-2003 (800 mg/kg) 03-MAR-2004 to 24-MAR-2004 (1 female, 2000 mg/kg) 05-MAR-2004 to 26-MAR-2004 (4 females, 2000 mg/kg) 09-MAR-2004 to 30-MAR-2004 (5 males, 2000 mg/kg)
Study Completion Date	07-JUN-2004

1.4 ARCHIVING

RCC Ltd (CH-4452 Ilingen / Switzerland) will retain the study plan, raw data, a sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

The remaining test item will be returned to the Sponsor. It is the Sponsor's responsibility to archive the test item.

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1.5 SIGNATURE PAGE

Study Director:

G. Arcelin

.....
date: 07-JUN-2004

Management:

for Dr. H. Fankhauser

.....
date: 07-JUN-2004

RCC STUDY NUMBER 851818
Study Reference Number KK030436

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Ittingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 851818
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Acute Dermal Toxicity Study in Rats

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures, with the exception of the trial formulation, were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
08-DEC-2003 Study Plan	08-DEC-2003
13-NOV-2003 Process Based (Test System, Test Item, Treatment, Raw Data)	13-NOV-2003
16-MAR-2004 Test System, Test Item, Treatment, Raw Data, Dose Preparation	16-MAR-2004
20-FEB-2004 Report 1	20-FEB-2004
04-JUN-2004 Report 2	04-JUN-2004

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

M. C. Schlepper

M. C. Schlepper
date: 07. Juni - 2004

RCC STUDY NUMBER 851818
Study Reference Number KK030436

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GOOD LABORATORY PRACTICE**1.7 STATEMENT OF COMPLIANCE**

RCC STUDY NUMBER : 851818
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Acute Dermal Toxicity Study in Rats

The supporting data for purity (characterisation), stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the Statement of Compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449. The solubility trials, to determine the choice of vehicle, were performed before the study initiation date and therefore are also excluded from this Statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director:

G. Arcelin

G. Arcelin
date: 07-JUN-2004

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Study Reference Number KK030436

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

OECD Guidelines for Testing of Chemicals, Section 4, Number 402 "Acute Dermal Toxicity", adopted February 24, 1987.

Directive 92/69/EEC, B.3. "Acute Toxicity-Dermal", July 31, 1992.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 32.

1.10 SUMMARY OF STUDY PLAN AMENDMENT

-Study plan Amendment No.1:

The test item was prepared incorrectly and therefore a dose of 800 mg/kg was dosed instead of a dose of 2000 mg/kg, as stated in the study plan. Based on the results from the 800 mg/kg dose group (see Section 9) it was decided that the study procedure should continue with a 2000 mg/kg dose, as originally planned, to enable an LD₅₀ value to be determined.

BCC STUDY NUMBER 851818
Study Reference Number KK030436

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2 SUMMARY OF RESULTS

[REDACTED] was dermally tested at 800 and 2000 mg/kg by using five male and five female HanBrl: WIST (SPF) rats per dose. The test item was diluted in vehicle (purified water) at a concentration of 0.2 or 0.5 g/mL, respectively and administered at a volume dosage of 4 mL/kg. The application period was 24 hours.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded, except in the five 2000 mg/kg treated males in which the mortality/viability and clinical signs were not recorded on the acclimatization day 4. All animals were examined for clinical signs at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded twice daily during test days 1-15, except in four 2000 mg/kg treated females in which the mortality/viability was recorded once on test day 1. Body weights were recorded on day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

No deaths occurred during the study.

When treated at 800 mg/kg, slight scaling was observed in one female at the 9- and 10-day reading and in one female between day 7 and the end of the study period (day 15). Two females were seen with focal erythema on the test site from the 7-day reading up to the end of the study period. No clinical signs were noted in the females. No clinical signs and no local signs were recorded in the males during the course of the study.

When treated at 2000 mg/kg, slight general erythema was observed in four of the females on test day 2 after removal of the dressing. One female still showed a slight erythema on test day 3. Slight focal erythema was seen in two females from test day 7 to the end of the study. No clinical signs were noted in the females. No clinical signs and no local signs were recorded in any of the males during the course of the study, however, a slight yellow discoloration was observed on the skin of all the males on day 2 which persisted in four of the animals for at least 3 days.

Loss of body weight (0.6 % to 4.9 %) was observed in one 800 mg/kg treated female and in three 2000 mg/kg females at the 8-day reading. By the end of the study, the animals had regained some of weight that was lost. In spite of this body weight loss, the body weight of all animals during the course of the study was within the range commonly recorded for this strain and age.

The caecum was distended with gas in one 800 mg/kg treated male at scheduled necropsy. There were no macroscopic findings in any of the other animals at scheduled necropsy.

3 CONCLUSION

The median lethal dose of [REDACTED] after single dermal administration to rats of both sexes, observed over a period of 14 days is:

LD₅₀ (rat): greater than 2000 mg/kg body weight

RCC STUDY NUMBER 851818
Study Reference Number KK030436

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4 PURPOSE

The purpose of this study was to assess the acute dermal toxicity of [REDACTED] when administered to rats by a single semi-occlusive dermal application, followed by an observation period of 14 days.

This study should provide a rational basis for risk assessment.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Rat, HanBrl: WIST (SPF)
Rationale	Recognized by the international guidelines as a recommended test system.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals per group	5 males and 5 females
Total number of animals	10 males and 10 females
Age when treated	Males: 9 weeks Females: 12 weeks
Identification	By unique cage number and corresponding color-coded spots on the tail. The animals were marked at acclimatization start.
Acclimatization	Under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

5.2 HUSBANDRY

Room no.	104 / RCC Ltd, Füllinsdorf
Conditions	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with target ranges for room temperature 22 ± 3 °C and for relative humidity between 30-70 % (values above 70 % during cleaning process possible), automatically controlled light cycle of 12 hours light and 12 hours dark, music during the daytime light period.

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Accommodation	During acclimatization in groups of four or five per sex or individually in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttlenz) during treatment and observation.
Diet	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 54/03 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) <i>ad libitum</i> . Results of analyses for contaminants are archived at RCC Ltd, Itingen.
Water	Community tap water from Füllinsdorf <i>ad libitum</i> . Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

5.3 TEST ITEM

The following information was provided by the sponsor:

Identity

Description

Sample number

S2539801

Purity

The supporting data for purity of the test item was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study Study Reference Number AC030449.

Expiry date

01-JAN-2005

Stability of test item dilution

Stability of the dosing solutions was addressed by ensuring fresh solutions were made immediately prior to dosing.

Storage conditions

At room temperature (range of 20 ± 3 °C), protected from light.

Safety precautions

Routine hygienic procedures were used to ensure the health and safety of the personnel.

The supporting data for stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study, Study Reference Number AC030449.

5.4 VEHICLE

The following information was provided by RCC Ltd:

Purified water prepared at RCC Ltd (deionised water which was processed and treated by the PURELAB Option-R unit. This latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).

A solubility trial was carried out to determine the choice of vehicle. This was a non-GLP trial, performed before the study initiation date, and therefore is excluded from the statement of compliance.

5.5 DOSE FORMULATION

The dose levels are in terms of the test item as supplied by the sponsor.

The test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added (weight:volume). The formulation was prepared shortly before the application using a magnetic stirrer and a spatula.

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

5.6 TREATMENT

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface.

Only those animals without injury or irritation on the skin were used in the test.

On test day 1, the test item was applied at a dose of 800 or 2000 mg/kg body weight evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage.

Application volume/kg body weight: 4 mL

Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.

The fur of the 800 mg/kg treated males was shaved on test day 7 (no. 1 to 4), test day 9 (no. 1 to 5), test day 11 (no. 1 to 5) and of the 800 mg/kg treated females on test day 9 (no. 6, 7, 10) just prior to the assessment of the reaction to facilitate the skin reading.

The fur of the 2000 mg/kg treated females was shaved on test day 3 and 8 (no. 11), test day 6 (no. 12 to 15), test day 8 and 14 (no. 13) and of the 2000 mg/kg treated males on test day 4 (no. 17, 18 and 20), test day 10 and 14 (no. 16 to 20).

Rationale: Dermal administration was used as this is one possible route of human exposure during manufacture, handling and use of the test item.

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5.7 OBSERVATIONS

Mortality / Viability	Daily during the acclimatization period except in the five 2000 mg/kg treated males (nos. 16-20) in which the mortality/viability was not recorded on the acclimatization day 4 and twice daily during days 1-15 except in four 2000 mg/kg treated females (nos. 12-15) in which the mortality/viability was recorded once on test day 1.
Body weights	On test days 1 (prior to administration), 8 and 15.
Clinical signs	Daily during acclimatization, except in the five 2000 mg/kg treated males (nos. 16-20) in which the clinical signs were not recorded on the acclimatization day 4, and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.

6 PATHOLOGY

6.1 NECROPSY

All animals were killed at the end of the observation period by an intraperitoneal injection of Vetanarcol at a dose of at least 2.0 mL/kg body weight (equivalent to at least 324 mg sodium pentobarbitone/kg body weight) and discarded after macroscopic examinations were performed. No organs or tissues were retained.

7 STATISTICAL ANALYSIS

No statistical analysis was used.

8 DATA COMPILATION

Body weights were recorded on-line.

Clinical signs were recorded on data sheets.

Mortality/viability were compiled into the RCC Tox Computer System during recording and/or recorded on data sheets.

Macroscopic findings were compiled into the RCC Tox Computer System during recording.

The RCC Tox Computer System (RCC-Tox-Lims) has been validated with respect to data collection, storage and retrievability.

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Study Reference Number KK030436

9 RESULTS

9.1 MORTALITY

No deaths occurred during the study.

9.2 CLINICAL / LOCAL SIGNS

Dose of 800 mg/kg:

Slight scaling was observed in one female at the 9- and 10-day reading and in one female between day 7 and the end of the study period (day 15). Two females were seen with focal erythema on the test site from the 7-day reading up to the end of the study period. No clinical signs were noted in the females. No clinical signs and no local signs were recorded in the males during the course of the study.

Dose of 2000 mg/kg:

Slight general erythema was observed in four of the females on test day 2 after removal of the dressing. One female still showed a slight erythema on test day 3. Slight focal erythema was seen in two females from test day 7 to the end of the study. No clinical signs were noted in the females. No clinical signs and no local signs were recorded in any of the males during the course of the study, however, a slight yellow discoloration was observed on the skin of all males on day 2 which persisted in four of the animals for at least 3 days.

9.3 BODY WEIGHTS

Loss of body weight (0.6 % to 4.9 %) was observed in one 800 mg/kg treated female (no. 9) and in three 2000 mg/kg females (nos. 11, 13 and 15) at the 8-day reading. By the end of the study, the animal had regained some of weight that was lost. In spite of this body weight loss, the body weight of all animals during the course of the study was within the range commonly recorded for this strain and age.

9.4 MACROSCOPIC FINDINGS

The caecum was distended with gas in one 800 mg/kg treated male at scheduled necropsy. There were no macroscopic findings in any of the other animals at scheduled necropsy.

9.5 MEDIAN LETHAL DOSE

The median lethal dose of [REDACTED] after single dermal administration to rats of both sexes, observed over a period of 14 days is:

LD₅₀ (rat): greater than 2000 mg/kg body weight

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 Study Reference Number KK030436

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10 INDIVIDUAL FINDINGS

10.1 CLINICAL / LOCAL SIGNS

Dose mg/kg	An. No.	Sex	Signs	Test days																	
				1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
				1*	2*	3*	5*														
800	1	M	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	2	M	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	3	M	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	4	M	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	5	M	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
800	6	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	7	F	Scaling											1	1						
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	8	F	Scaling										1	1	1	1	1	1	1	1	1
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	9	F	Focal erythema										1	1	1	1	1	1	1	1	1
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	10	F	Focal erythema										1	1	1	1	1	1	1	1	1
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Key: 1 slight, √ noted

* Examinations were performed approximately 1, 2, 3 and 5 hours after treatment.

No clinical signs were evident in any animal during the acclimatization period.

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Dose mg/kg	An. No.	Sex	Signs	Test days																	
				1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
				1*	2*	3*	5*														
2000	11	F	General erythema					1													
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	12	F	General erythema					1													
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	13	F	General erythema					1	1												
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	14	F	General erythema					1													
			Focal erythema										1	1	1	1	1	1	1	1	1
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	15	F	Focal erythema									1	1	1	1	1	1	1	1	1	1
No clinical signs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
2000	16	M	Yellow discoloration					1	1	1	1										
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	17	M	Yellow discoloration					1	1	1											
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	18	M	Yellow discoloration					1	1	1											
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	19	M	Yellow discoloration					1	1	1	1	1	1	1							
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	20	M	Yellow discoloration					1													
			No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Key: 1 slight, √ noted

* Examinations were performed approximately 1, 2, 3 and 5 hours after treatment.

No clinical signs were evident in any animal during the acclimatization period.

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10.2 BODY WEIGHTS

Body weight in grams:				
Sex / Dose	Animal No.	Day of Treatment	Day 8	Day 15
Male / 800 mg/kg	1	245.8	262.9	287.6
	2	255.9	278.0	304.4
	3	263.0	284.8	312.7
	4	272.9	299.5	329.4
	5	281.2	284.5	308.8
Female / 800 mg/kg	6	250.9	247.8	256.8
	7	244.0	247.8	250.3
	8	252.6	252.6	262.7
	9	245.5	233.5	240.1
	10	240.2	240.4	256.3
Female / 2000 mg/kg	11	234.5	233.1	243.5
	12	252.1	255.5	265.8
	13	247.1	244.6	266.8
	14	252.4	259.7	269.4
	15	229.7	226.8	240.3
Male / 2000 mg/kg	16	260.7	287.3	313.3
	17	279.4	297.9	334.6
	18	268.1	281.7	303.5
	19	261.9	278.2	293.5
	20	258.2	281.0	303.2

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10.3 MACROSCOPIC FINDINGS

Sex / Dose	Animal No.	Mode of Death	Findings
Male / 800 mg/kg	1	Scheduled necropsy	No macroscopic findings.
	2	Scheduled necropsy	No macroscopic findings.
	3	Scheduled necropsy	Caecum distended with gas.
	4	Scheduled necropsy	No macroscopic findings.
	5	Scheduled necropsy	No macroscopic findings.
Female / 800 mg/kg	6	Scheduled necropsy	No macroscopic findings.
	7	Scheduled necropsy	No macroscopic findings.
	8	Scheduled necropsy	No macroscopic findings.
	9	Scheduled necropsy	No macroscopic findings.
	10	Scheduled necropsy	No macroscopic findings.
Female / 2000 mg/kg	11	Scheduled necropsy	No macroscopic findings.
	12	Scheduled necropsy	No macroscopic findings.
	13	Scheduled necropsy	No macroscopic findings.
	14	Scheduled necropsy	No macroscopic findings.
	15	Scheduled necropsy	No macroscopic findings.
Male / 2000 mg/kg	16	Scheduled necropsy	No macroscopic findings.
	17	Scheduled necropsy	No macroscopic findings.
	18	Scheduled necropsy	No macroscopic findings.
	19	Scheduled necropsy	No macroscopic findings.
	20	Scheduled necropsy	No macroscopic findings.

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11 GLP – CERTIFICATION

The Swiss GLP Monitoring Authorities

Swiss Federal
Office of
Public HealthSwiss Agency for the
Environment, Forests
and Landscape

swissmedic

Swissmedic
Swiss Agency for
Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of

November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Ittingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities**Areas of expertise ***

- Toxicology

TOX, ACC, MUT,
OTH (Safety Pharmacology)- Environmental Chemistry and
PharmaceuticalsACC, ECT, ENF, EMN, PCT,
RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology; ACC = Analytical and Clinical Chemistry; ECT = Environmental toxicity on aquatic and terrestrial organisms; ENF = Behaviour in water, soil and air; Bioaccumulation; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing; RES = Residue studies; OTH = Other, to be specified.

RCC Study Number 851879

[REDACTED] Study Reference Number KSI030435

[REDACTED]
Primary Skin Irritation Study in Rabbits
(4-Hour Semi-Occlusive Application)

Report

Author: G. Arcelin

Sponsor: [REDACTED]

RCC STUDY NUMBER 851879
Study Reference Number KSI030435

Report

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1. PREFACE

1.1 GENERAL

Title

[REDACTED]
Primary Skin Irritation Study in Rabbits
(4-Hour Semi-Occlusive Application)

Sponsor

[REDACTED] Project Planning
Contact Names

Mrs L. Selbie
Mrs C. Talbot
Miss J. Evans

Scientific Representative

Miss K. Wilson

Test Facility

RCC Ltd
Toxicology
Operational Unit: Safety Assessment I
Wölferstrasse 4
CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	G. Arcelin
Deputy Study Director	M. Ott
Technical Coordinator	P. Reissbrodt
Head of RCC Quality Assurance	I. Wüthrich


1.3 SCHEDULE

Experimental Starting Date	06-JAN-2004
Experimental Completion Date	15-JAN-2004
Acclimatization	06-JAN-2004 to 11-JAN-2004
Treatment	12-JAN-2004
Observation of local findings	Throughout 72 hours after treatment.
Termination	15-JAN-2004
Study Completion Date	05-APR-2004

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1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

The remaining test item will be returned to the Sponsor. Archiving of the test item is the responsibility of the Sponsor.

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1.5 SIGNATURES

Study Director:

G. Arcelin

G. Arcelin
date: 05-APR-2004

Management:

(for) Dr. H. Fankhauser

H. Fankhauser
date: 01-APR-2004

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Illingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 851879
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcellin
TITLE : [REDACTED]
Primary Skin Irritation Study in Rabbits
(4-Hour Semi-Occlusive Application)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures, with the exception of the pH measurement of the test item, were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
23-DEC-2003 Study Plan	23-DEC-2003
26-JAN-2004 Process Based (Test System, Test Item, Treatment, Raw Data)	26-JAN-2004
17-MAR-2004 Report	17-MAR-2004

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

(for) G. Hohl

L. C. Lechner
date: 05. April 2004

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Study Reference Number KSI030435

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GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER : 851879
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Primary Skin Irritation Study in Rabbits
(4-Hour Semi-Occlusive Application)

The supporting data for purity (characterisation) of the test item were not made available at the time of issuing this report and hence this information has been excluded from the Statement of Compliance. However, the sponsor has addressed this in a GLP compliant study, Study Reference Number AC030449.

The pH measurement of the test item was performed before the study initiation date. This procedure is, therefore, excluded from this statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 25th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director:

G. Arcelin


date: 05-APR-2004

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Study Reference Number KSI030435

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

Directive 92/69 EEC, B.4. "Acute Toxicity - Skin Irritation", July 31, 1992.

OECD Guidelines for Testing of Chemicals, Section 4, number 404 "Acute Dermal Irritation / Corrosion", adopted April 24, 2002.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 55.

1.10 CLASSIFICATION GUIDELINES

Commission Directive 2001/59/EC adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, August 06, 2001 (Official Journal of the European Communities Nr. L 225/1, August 21, 2001).

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Study Reference Number KSI030435

2. SUMMARY

The primary skin irritation potential of [REDACTED] was investigated according to OECD test guideline no. 404. The test item was applied by topical semi-occlusive application of 0.5 g to the intact left flank of each of three young adult New Zealand White rabbits. The duration of treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing.

The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. The mean erythema/eschar score and the mean oedema score were 0.00 for all three animals.

The application of [REDACTED] to the skin resulted in mild signs of irritation (very slight erythema), in two animals. This effect was reversible and was no longer evident 24 hours after treatment. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no other clinical signs of test item related effects were observed.

Thus, the test item did not induce significant or irreversible damage to the skin.

Based upon the referred classification criteria (Commission Directive 2001/59/EC of August 2001), [REDACTED] is considered to be "not irritating" to rabbit skin.

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3. PURPOSE

The purpose of this primary skin irritation study was to assess the possible irritation potential when a single dose of [REDACTED] was placed on the skin of rabbits for approximately four hours.

This study should provide a rational basis for risk assessment in man as skin contact is one of the possible routes of human exposure.

The test item was administered at 0.5 g/animal, the dose specified in the test guidelines for a solid test item.

4. MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	New Zealand White Rabbit, SPF
Rationale	Recognized by the international guidelines as the recommended test system.
Source	Elevage Scientifique des Dombes F-01400 Chatillon sur Chalaronne / France
Number of animals per test	3 (Animals of both sexes were used)
Age at treatment	11 - 12 weeks (male) 11 - 12 weeks (females)
Identification	By unique cage number and corresponding ear number.
Acclimatization	Under laboratory conditions after health examination. Only animals without any visual signs of illness were used for the study.
Allocation	Male No. 21 Female Nos. 22 and 23

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Study Reference Number KSI030435

4.2 HUSBANDRY

Room number

106 / RCC Ltd, Füllinsdorf

Conditions

Standard Laboratory Conditions

Air-conditioned with target ranges for room temperature 17-23 °C, relative humidity 30-70 % and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges may have occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the daytime light period.

Accommodation

Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Wood blocks (RCC Ltd, Füllinsdorf) and haysticks 4646 (batch no. 0303 Provimi Kliba AG) were provided for gnawing.

Diet

Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum* (batch no. 63/03) provided by Provimi Kliba AG, CH-4303 Kaiseraugst. Results of analysis for contaminants are archived at RCC Ltd, Itingen.

Water

Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

4.3 TEST ITEM

The following information was provided by the Sponsor:

Identification

Description

sample number

S2539801

Purity

The supporting data for purity of the test item was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study, SEAC Study Reference Number AC030449.

Expiry date

01-JAN-2005

Storage conditions

At room temperature (range of 20 ± 3 °C), protected from light.

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Study Reference Number KSI030435

Safety precautions

Routine hygienic procedures were used to ensure the health and safety of the personnel.

4.4 TEST ITEM PREPARATION

0.5 g (per animal) of [REDACTED] was weighed as delivered by the Sponsor and then moistened with approximately 0.1 mL of purified water before application.

The pH of the test item was measured before the study initiation date. A formulation of 1 % in water was prepared. The pH was found to be 5.

According to Directive 92/69 EEC, B.4. and OECD Guidelines 404, a test item needs not to be tested if the pH-value is less than 2 or greater than 11.5, owing to its predictable corrosive properties.

4.5 TREATMENT

Four days before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 100 cm² (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again.

Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of [REDACTED] was placed on a surgical gauze patch (ca. 4 cm x 4 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape.

The duration of treatment was 4 hours. Then the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

4.6 OBSERVATIONS

Viability/Mortality	Daily from acclimatization of the animals to the termination of test.
Clinical signs	Daily from acclimatization of the animals to the termination of test.
Body weights	At start of acclimatization, on the day of application and at termination of observation.

4.7 IRRITATION SCORES

The skin reaction was assessed according to the numerical scoring system listed in the EEC Commission Directive 92/69/EEC, July 31, 1992 approximately 1, 24, 48 and 72 hours after the removal of the dressing, gauze patch and test item.

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4.8 TREATMENT OF RESULTS

Data are summarized in tabular form, showing the irritation scores for erythema and oedema for each individual animal at all observation intervals after patch removal, any lesions, a description of the degree and nature of irritation, corrosion or reversibility, and any other toxic effects observed.

To evaluate the irritation of the test item the mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. An animal is positive when the mean score is 2 or greater. The test is positive for irritation when at least 2 animals are positive for the same endpoint (erythema/eschar or oedema).

5. PATHOLOGY

5.1 NECROPSY

No necropsy was performed on the animals sacrificed at termination of observation.

All rabbits were sacrificed by an intravenous injection of Vetanarcol into the ear vein at a dose of at least 1 mL/kg body weight (equivalent to 162 mg sodium pentobarbitone/kg body weight) and discarded.

6. DATA COMPILATION AND STATISTICAL ANALYSIS

Viability/mortality, clinical signs and dermal findings were recorded on data sheets and transcribed for compilation and analysis.

Body weights were recorded on-line.

No statistical analysis was performed.

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7. RESULTS

7.1 VIABILITY/MORTALITY/CLINICAL SIGNS

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

7.2 IRRITATION

The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. The mean erythema/eschar score and the mean oedema score were 0.00 for all three animals.

Very slight erythema was observed in two animals at the 1-hour reading.

No abnormal findings were observed on the treated skin of any animal 24 hours after treatment.

7.3 COLORATION

No staining produced by the test item of the treated skin was observed.

7.4 CORROSION

Neither alterations of the treated skin were observed nor were corrosive effects evident on the skin.

7.5 BODY WEIGHTS

The body weights of all rabbits were considered to be within the normal range of variability.

Body weight in grams				
Animal No.	Sex	First Day of Acclimatization	Day of Treatment	Last Day of Observation
21	male	1994	2195	2219
22	female	2063	2046	2156
23	female	2029	2244	2315

7.6 CONCLUSION

Based upon the referred classification criteria (Commission Directive 2001/59/EC of August 2001) [REDACTED] is considered to be "not irritating" to rabbit skin.

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8. APPENDICES

8.1 SKIN IRRITATION SCORES

Key to Symbols:

M Male

F Female

Note: EEC Commission Directive 92/69/EEC, July 31, 1992, Grading of Skin Reactions is presented on page 21.

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TABLE 1: SKIN IRRITATION SCORES - INDIVIDUAL VALUES

Animal Number	Sex	Evaluation Interval*	Erythema	Oedema
21	M	1 hour	1	0
22	F		1	0
23	F		0	0
21	M	24 hours	0	0
22	F		0	0
23	F		0	0
21	M	48 hours	0	0
22	F		0	0
23	F		0	0
21	M	72 hours	0	0
22	F		0	0
23	F		0	0

* Examinations were performed at the specified times after removal of the dressing.

TABLE 2: SKIN IRRITATION SCORES – INDIVIDUAL MEAN VALUES AFTER 24, 48 AND 72 HOURS

Animal Number	Sex	Erythema	N	Oedema	N
21	M	0.00	3	0.00	3
22	F	0.00	3	0.00	3
23	F	0.00	3	0.00	3

N=number of available data points.

TABLE 3: SKIN IRRITATION SCORES – ASSESSMENT ACCORDING TO EEC GUIDELINES

Evaluated intervals	Erythema	Oedema
24 hours	Not Irritating	Not Irritating
48 hours		
72 hours		

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Study Reference Number KSI030435

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8.2 INDIVIDUAL FINDINGS

ANIMAL NO. 21, MALE

After 1 hour:	Erythema:	very slight erythema
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

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INDIVIDUAL FINDINGS**ANIMAL NO. 22, FEMALE**

After 1 hour:	Erythema:	very slight erythema
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

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INDIVIDUAL FINDINGS**ANIMAL NO. 23, FEMALE**

After 1 hour:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Oedema:	NO ABNORMAL FINDINGS NOTED
	Flaking:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

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Study Reference Number KSI030435

8.3 SUMMARY OF EVALUATION CRITERIA

- EEC Commission Directive 92/69/EEC, July 31, 1992
- Commission Directive 2001/59/EC, August 2001

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Study Reference Number KSI030435

EEC COMMISSION DIRECTIVE 92/69/EEC, JULY 31, 1992

Grading of Skin Reactions

ERYTHEMA AND ESCHAR FORMATION

No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema.....	3
Severe erythema (beet redness) or eschar formation (injuries in depth preventing erythema) reading	4

OEDEMA FORMATION

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (edges raised approximately 1 mm).....	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)....	4

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Commission Directive 2001/59/EC, August 2001

The following risk phrase shall be assigned in accordance with the criteria given:

"R38 Irritating to skin"

Substances and preparations which cause significant inflammation of the skin which persists for at least 24 hours after an exposure period of up to four hours determined on the rabbit according to the cutaneous irritation test method cited in Annex V.

Inflammation of the skin is significant if:

- (a) the mean value of the scores for either erythema and eschar formation or oedema formation, calculated over all the animals tested, is 2 or more, or
- (b) in the case where the Annex V test has been completed using three animals, either erythema and eschar formation or oedema formation equivalent to a mean value of 2 or more calculated for each animal separately has been observed in two or more animals.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time. Particular effects e.g. hyperplasia, scaling, discoloration, fissures, scabs and alopecia should be taken into account.

Substances and preparations which cause significant inflammation of the skin, based on practical observation in humans on immediate, prolonged or repeated contact.

Organic peroxides, except where evidence to the contrary is available.

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8.4 GLP - CERTIFICATION

The Swiss GLP Monitoring Authorities



Swiss Federal
Office of
Public Health



Swiss Agency for the
Environment, Forests
and Landscape



SWISSmedic
Swiss Agency for
Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of

November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities

Areas of expertise *

- Toxicology

TOX, ACC, MUT,
OTH (Safety Pharmacology)

- Environmental Chemistry and Pharmanalytics

ACC, ECT, ENF, EMN, PCT,
RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air, Bioaccumulation ; EMN = Studies on effects on mesocosms and natural ecosystems ; MUT = Mutagenicity ; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.

RCC Study Number 851880

[REDACTED] Study Reference Number KIE030434

[REDACTED]

Primary Eye Irritation Study in Rabbits

Report

Author: G. Arcelin

Sponsor: [REDACTED]

RCC STUDY NUMBER 851880
Study Reference Number KIE030434

Report

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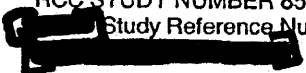
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Study Reference Number K1E030434

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1 PREFACE

1.1 GENERAL

Title

Primary Eye Irritation Study in Rabbits

Sponsor

Project Planing
Contact Names

Mrs L. Selbie
Mrs C. Talbot
Mrs T. Gübler
Miss J. Evans

Scientific Representative

Miss K. Wilson

Test Facility

RCC Ltd
Toxicology
Operational Unit: Safety Assessment I
Wölferstrasse 4
CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	G. Arcelin
Deputy for Study Director	M. Ott
Technical Coordinator	P. Reissbrodt
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date	27-JAN-2004
Experimental Completion Date	02-MAR-2004
Acclimatization	27-JAN-2004 to 01-FEB-2004 (one female) 27-JAN-2004 to 02-FEB-2004 (one male and one female) 24-FEB-2004 to 01-MAR-2004 (one female)
Treatment	02-FEB-2004 (one female) 03-FEB-2004 (one male and one female) 02-MAR-2004 (one female)

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[REDACTED] Study Reference Number KIE030434

Observation of local findings	Throughout 72 hours after treatment.
Termination	02-MAR-2004
Study Completion Date	26-MAY-2004

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, amendments, raw data, a sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

The remaining test items will be returned to the Sponsor. Archiving of the test items is the responsibility of the Sponsor.

RCC STUDY NUMBER 851880
Study Reference Number KIE030434

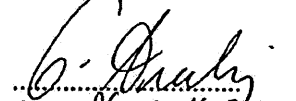
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1.5 SIGNATURE PAGE

Study Director:

G. Arcelin


date: 26-MAY-2004

Management:

(for) Dr. H. Fankhauser


date: 25-MAY-2004

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Study Reference Number KIE030434

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 851880
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Primary Eye Irritation Study in Rabbits

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
23-JAN-2004 Study Plan	23-JAN-2004
26-JAN-2004 Process Based (Test System, Test Item, Treatment, Raw Data, Dose Preparation)	26-JAN-2004
14-MAY-2004 Report	14-MAY-2004

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

(for) S. van Dongen

L. C. Schlepper
date: 26. Mai - 2004

RCC STUDY NUMBER 851880
[REDACTED] Study Reference Number KIE030434

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GOOD LABORATORY PRACTICE**1.7 STATEMENT OF COMPLIANCE**

RCC STUDY NUMBER : 851880
TEST ITEM : [REDACTED]
STUDY DIRECTOR : G. Arcelin
TITLE : [REDACTED]
Primary Eye Irritation Study in Rabbits

The supporting data for purity (characterisation), stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the Statement of Compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director:

G. Arcelin

G. Arcelin
date: 26 - MAY - 2004

RCC STUDY NUMBER 851880

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Study Reference Number KIE030434

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

Directive 92/69 EEC, B.5. "Acute Toxicity - Eye Irritation", July 31, 1992.

OECD Guidelines for Testing of Chemicals, Section 4, number 405 "Acute Eye Irritation / Corrosion", adopted April 24, 2002.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 54.

1.10 CLASSIFICATION GUIDELINES

Commission Directive 2001/59/EC adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, August 06, 2001 (Official Journal of the European Communities Nr. L 225/1, August 21, 2001).

1.11 SUMMARY OF STUDY PLAN AMENDMENTS

Study Plan Amendment No. 1:

Re-definition of the necropsy procedure.

Study Plan Amendment No. 2:

Completion of the study with an additional animal, due to the spontaneous death of one of the three animals.

RCC STUDY NUMBER 851880

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Study Reference Number KIE030434

2 SUMMARY

The primary eye irritation potential of [REDACTED] was investigated according to OECD test guideline no. 405. The test item was applied by instillation of 0.1 mL of [REDACTED] (corresponding to 0.03 g of test item) into the left eye of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours after test item instillation. As one female rabbit was found dead at the 1-hour reading, a further female was treated under the same conditions to complete the study to three animals. Unfortunately, the same phenomenon occurred, the additional animal was found dead approximately 40 minutes after the test item instillation. No conclusion was reached concerning the cause of spontaneous death in these two animals.

The instillation of [REDACTED] into the eyes of the two surviving rabbits resulted in mild, early-onset and transient ocular changes in one animal only (female). These changes were reversible and were no longer evident 48 hours after treatment. In the second animal (male) no abnormal findings were noted, at any of the assessment times. No corrosion or staining of the treated eyes was observed in either of these animals.

Thus, the test item did not induce significant or irreversible damage to the rabbit eye.

The study was closed after the 72-hour reading.

Based upon the referred classification criteria (Commission Directive 2001/59/EC of August 06, 2001), [REDACTED] is considered to be "not irritating" to the rabbit eye.

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Study Reference Number KIE030434

3 PURPOSE

The purpose of this primary eye irritation study was to assess the possible irritation potential when a single dose of [REDACTED] was placed in the conjunctival sac of rabbit eyes.

This study should provide a rational basis for risk assessment in man as ocular contact is one of the possible routes of human exposure.

The test item was applied as a volume of 0.1 mL (equivalent to approximately 0.03 g, according to the Sponsor information).

4 MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	New Zealand White Rabbit, SPF
Rationale	Recognized by the international guidelines as the recommended test system.
Source	Elevage Scientifique des Dombes F-01400 Chatillon sur Chalaronne / France
Number of animals per test	4* (Animals of both sexes were used)
Age at treatment	12 - 13 weeks (male) 11 - 13 weeks (females)
Identification	By unique cage number and corresponding ear number.
Acclimatization	Under laboratory conditions after health examination. Only animals without any visual signs of illness were used for the study.
Allocation	Male No. 24 Female Nos. 25 to 27

* Three is the number of animals required for this study type. As death occurred in one female (no. 26), a further animal (no. 27) was treated.

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4.2 HUSBANDRY

Room number

106 / RCC Ltd, Füllinsdorf

Conditions

Standard Laboratory Conditions

Air-conditioned with target ranges for room temperature 17-23 °C, relative humidity 30-70 % and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges may have occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the daytime light period.

Accommodation

Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Wood blocks (RCC Ltd, Füllinsdorf) and haysticks 4646 (batch no. 0403, Provimi Kliba AG) were provided for gnawing.

Diet

Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum* (batch no. 86/03) provided by Provimi Kliba AG, CH-4303 Kaiseraugst. Results of analysis for contaminants are archived at RCC Ltd, Itingen.

Water

Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

4.3 TEST ITEM

The following information was provided by the Sponsor:

Identification

Description

[REDACTED] sample number

S2539801

Purity

The supporting data for purity of the test item was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

Expiry date

01-JAN-2005

Storage conditions

At room temperature (range of 20 ± 3 °C), protected from light.

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Study Reference Number KIE030434

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Safety precautions

Routine hygienic procedures were used to ensure the health and safety of the personnel.

The supporting data for stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

4.4 TEST ITEM PREPARATION

0.03 g (equivalent to 0.1 mL) of [REDACTED] was weighed (per animal) and applied undiluted as it was delivered by the Sponsor.

The pH of a 1% (w/w) solution of the test item was measured for a previous study (RCC Study number 851879, skin irritation with [REDACTED] in rabbits) and was found to be 5.

According to Directive 92/69 EEC, B.5. and OECD Guidelines 405, a test item needs not to be tested if the pH-value is less than 2 or greater than 11.5, owing to its predictable corrosive properties.

4.5 TREATMENT

The eyes of the animals were examined one day prior to test item administration.

Animals with overt signs of ocular injury or irritation which may have interfered with the interpretation of the results were not used in the test.

The test item was flocculated (loosely packed) and therefore the volume of 0.1 g of the test item was too large to be administered into the conjunctival sac of the animals. Accordingly a dose volume of 0.1 mL was chosen. This had a weight of approximately 0.03 g.

On the day of treatment 0.1 mL (equivalent to 0.03 g) of [REDACTED] was placed in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The right eye remained untreated and served as the reference control. The treated eyes were not rinsed after instillation.

A single animal (one female) was treated first. As neither a corrosive effect nor a severe irritant effect was observed after the 1- and 24-hour examinations, the test was completed using the two remaining animals (one male and one female). As death occurred in the second female, a further female was treated in the same conditions. A total of four animals were used for this study.

4.6 OBSERVATIONS**Viability/Mortality**

Daily from acclimatization of the animals to the termination of test.

Clinical signs

Daily from acclimatization of the animals to the termination of test.

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Body weights

At start of acclimatization, on the day of application and at termination of observation.

4.7 IRRITATION SCORES

The ocular reaction was assessed according to the numerical scoring system listed in the EEC Commission Directive 92/69/EEC, July 31, 1992 at approximately 1, 24, 48 and 72 hours after instillation.

When present, corrosion and/or staining of conjunctivae, sclerae and cornea by the test item were recorded and reported.

Eye examinations were made with a Varta Cliptrix diagnostic-lamp (Roth AG, CH-4153 Reinach/Switzerland).

4.8 TREATMENT OF RESULTS

Data are summarized in tabular form, showing the irritation scores of each following parameters: corneal opacity (including the area affected, where applicable), iridic effects, chemosis, conjunctival and scleral reddening for each individual animal at all observation intervals. In addition, any lesions including the degree and nature of irritation, corrosion or reversibility, and any other toxic effects are presented. As death occurred and the value of only two animals were available, mean values were not calculated.

For EU Classification of ocular irritants (Commission Directive 2001/59/EC), the criteria from the Official Journal of the European Communities (O.J. L 225/1) was employed (see page 23).

5 PATHOLOGY

5.1 NECROPSY

Both females which died after the test item instillation were necropsied as soon as they were found dead and any abnormalities were recorded.

At termination of observation the surviving animals were killed by intravenous injection of Vetanarcol into the ear vein at a dose of at least 1 mL/kg body weight (equivalent to 162 mg sodium pentobarbitone/kg body weight) and necropsy was performed.

6 DATA COMPILATION AND STATISTICAL ANALYSIS

Viability/mortality and ocular findings were recorded on data sheets and transcribed for compilation and analysis. The macroscopic findings were recorded on data sheets or compiled into the RCC Tox Computer System. Body weights were recorded on-lie.

No statistical analysis was performed. The RCC Tox Computer System (RCC-Tox-Lims) has been validated with respect to data collection, storage and retrievability.

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Study Reference Number KIE030434

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7 RESULTS

7.1 VIABILITY/MORTALITY AND CLINICAL SIGNS

Two females treated in this study died after test item instillation. The first animal was found dead one hour after treatment and the second animal was found dead 40 minutes after treatment. Both females which died after the test item instillation were necropsied as soon as they were found dead and any abnormalities were recorded. No clinical signs were observed immediately after the treatment. The animals were not continuously observed before the 1-hour reading as no death and no clinical signs were expected. No clinical signs were observed in the two surviving animals.

7.2 MACROSCOPIC FINDINGS AT NECROPSY

No macroscopic findings were noted in the two surviving animals at the end of the study.

Several pale foci were seen in the heart of the first female (no. 26) which was found dead one hour after the test item instillation.

The lungs of the additional female which was found dead approximately 40 minutes after the test item instillation were dark-red discolored.

No conclusion was reached concerning the cause of spontaneous death in these two animals.

7.3 IRRITATION

In the two surviving rabbits (one male and one female), only the female showed ocular changes such as a slight opacity in the whole corneal area, a moderate redness of the conjunctivae and sclera and a moderate chemosis at the 1-hour reading. A slight redness of the conjunctivae and sclera was still observed at the 24-hour reading. These effects were reversible and were no longer evident 48 hours after treatment. In the second animal (male) no abnormal findings were noted, at any of the assessment times.

The study was closed after the 72-hour reading.

7.4 COLORATION

No staining of the treated eyes produced by the test item was observed.

7.5 CORROSION

No corrosion of the cornea was observed at any of the reading times.

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[REDACTED] Study Reference Number KIE030434

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7.6 BODY WEIGHTS

The body weights of all rabbits were considered to be within the normal range of variability.

Body weight in grams				
Animal No.	Sex	First Day of Acclimatization	Day of Treatment	Last Day of Observation
24	male	2121	2409	2497
25	female	2032	2244	2389
26*	female	2121	2318	-
27*	female	2143	2270	-

* one female rabbit (no. 26) was found dead 1 hour after the test item instillation. A further female (no. 27) was treated in the same conditions to complete the study to three animals. Unfortunately, the same phenomenon occurred, the additional animal was found dead approximately 40 minutes after the test item instillation.

7.7 CONCLUSION

Based upon the referred classification criteria (Commission Directive 2001/59/EC of August 06, 2001) [REDACTED] is considered to be "not irritating" to the rabbit eye.

RCC STUDY NUMBER 851880
Study Reference Number KIE030434

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8 APPENDICES

8.1 EYE IRRITATION SCORES

Key to Symbols:

M Male

F Female

Note: EEC Commission Directive 92/69/EEC, July 31, 1992, Grading of Ocular Lesions is presented on page 22 and used for classification under the Commission Directive 2001/59/EC, August 06, 2001 on page 23.

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TABLE 1: EYE IRRITATION SCORES - INDIVIDUAL VALUES

Animal Number	Sex	Evaluation Interval*	Corneal Opacity	Area of Corneal Opacity	Iris	Conjunctivae		Sclera
						Redness	Chemosis	
24	M	1 hour	0	0	0	0	0	0
25	F		1	4	0	2	2	2
24	M	24 hours	0	0	0	0	0	0
25	F		0	0	0	1	0	1
24	M	48 hours	0	0	0	0	0	0
25	F		0	0	0	0	0	0
24	M	72 hours	0	0	0	0	0	0
25	F		0	0	0	0	0	0

* Examinations were performed at the specified times after instillation of the test item.

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Study Reference Number KIE030434

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8.2 INDIVIDUAL FINDINGS

ANIMAL NO. 24, MALE

After 1 hour:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 24 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 48 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 72 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane), sclera and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, oedema, discharge, staining and test item remnants were assessed.

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Study Reference Number KIE030434

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INDIVIDUAL FINDINGS**ANIMAL NO. 25, FEMALE**

After 1 hour:	Cornea:	slight opacity, whole corneal area
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	moderately reddened; moderate swelling
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	moderately reddened
	Test item:	NO REMNANTS EVIDENT
After 24 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	slightly reddened
	Test item:	NO REMNANTS EVIDENT
After 48 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 72 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Sclerae:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane), sclera and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, oedema, discharge, staining and test item remnants were assessed.

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8.3 SUMMARY OF EVALUATION CRITERIA

- EEC Commission Directive 92/69/EEC, July 31, 1992
- Commission Directive 2001/59/EC, August 06, 2001

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EEC COMMISSION DIRECTIVE 92/69/EEC, JULY 31, 1992

Grading of Ocular Lesions

CORNEA

Opacity: degree of density (area most dense taken for reading)	
No ulceration or opacity.....	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible.....	3
Opaque cornea, iris not discernible through the opacity	4
Area of cornea involved	
Zero	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

IRIS

Normal.....	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these).....	2

CONJUNCTIVAE

Redness (refers to most severe reading of palpebral and bulbar conjunctivae when compared with control eye)	
Blood vessels normal.....	0
Some blood vessels definitely hyperemic (injected).....	1
Diffuse, crimson color, individual vessels not easily discernible	2
Diffuse beefy red	3
Chemosis: lids and/or nictitating membranes	
No swelling	0
Any swelling above normal (including nictitating membranes).....	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed.....	4
Discharge:	
No discharge	0
Any amount different to normal (does not include small amount observed in inner canthus of normal animal)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and hairs, and a considerable area around the eye (running)	3

Note: Reddening of the sclerae will be assessed using the same scoring grades as conjunctivae.

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COMMISSION DIRECTIVE 2001/59/EC, AUGUST 06, 2001

The following risk phrases shall also be assigned in accordance with the criteria given:

"R36 - Irritating to eyes"

Substances and preparations which, when applied to the eye of the animal, cause significant ocular lesions which occurred within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test cited in Annex V have any of the following values:

- cornea opacity equal to or greater than 2 but less than 3;
- iris lesion equal to or greater than 1 but not greater than 1,5;
- redness of the conjunctivae equal to or greater than 2,5;
- oedema of the conjunctivae (chemosis) equal to or greater than 2;

or, in the case where the Annex V test has been completed using three animals if the lesions, on two or more animals, are equivalent to any of the above values except that for iris lesion the value should be equal to or greater than 1 but less than 2 and for redness of the conjunctivae the value should be equal to or greater than 2,5.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Substances or preparations which cause significant ocular lesions, based on practical experience in humans.

Organic peroxides except where evidence to the contrary is available.

"R41 - Risk of serious damage to eyes"

Substances and preparations which, when applied to the eye of the animal, cause severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are severe if the mean scores of the eye irritation test in Annex V have any of the following values:

- cornea opacity equal to or greater than 3;
- iris lesion greater than 1,5.

The same shall be the case where the test has been completed using three animals if the lesion, on two or more animals, have any of the values:

- cornea opacity equal to or greater than 3;
- iris lesion equal to 2.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Ocular lesions are also severe when they are still present at the end of the observation time.

Ocular lesions are also severe if the substance or preparation causes irreversible colouration of the eyes.

Substances and preparations which cause severe ocular lesions, based on practical experience in humans.

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8.4 GLP - CERTIFICATION

The Swiss GLP Monitoring Authorities



Swiss Federal
Office of
Public Health



Swiss Agency for the
Environment, Forests
and Landscape

swissmedic

Swissmedic
Swiss Agency for
Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of

November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities**Areas of expertise *****- Toxicology**

TOX, ACC, MUT,
OTH (Safety Pharmacology)

**- Environmental Chemistry and
Pharmanalytics**

ACC, ECT, ENF, EMN, PCT,
RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology; ACC = Analytical and Clinical Chemistry; ECT = Environmental toxicity on aquatic and terrestrial organisms; ENF = Behaviour in water, soil and air; Bioaccumulation; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing; RES = Residue studies; OTH = Other, to be specified.

RCC Study Number 851904

Sponsor's Reference Number KSL030433



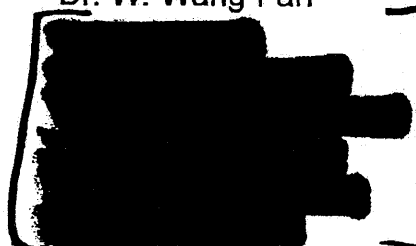
**Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)**

Report

Author:

Dr. W. Wang-Fan

Sponsor:



Study Completion Date: 02 April 2004

Total Number of Pages: 45

RCC STUDY NUMBER 851904
 Sponsor's Reference Number KSL030433
 [REDACTED]

REPORT

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REPORT

1 PREFACE

1.1 GENERAL

Title

[REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

Sponsor

Scientific Representative

[REDACTED] Contacts

[REDACTED]

RCC STUDY NUMBER 851904
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Test Facility	a) RCC Ltd Toxicology Operational Unit: Safety Assessment I CH - 4452 Itingen / Switzerland
Test Site	b) RCC Ltd Environmental Chemistry & Pharamanalytics CH - 4452 Itingen / Switzerland
Lead QA	RCC Ltd Quality Assurance GLP Toxicology CH - 4452 Itingen / Switzerland
Test Site QA	RCC Ltd Quality Assurance GLP Environmental Chemistry & Pharamanalytics CH - 4452 Itingen / Switzerland (Responsible for test site)

1.2 RESPONSIBILITIES

Study Director	Dr. W. Wang-Fan (a)
Deputy Study Director	L.G. Ullmann (a)
Technical Coordinator / Necropsy	N. Schäfer (a)
Head of Lead Quality Assurance	I. Wüthrich

Principal Investigator

Study Phase: ³HTdR Determination Dr. R. Burri (b)

1.3 SCHEDULE

Experimental Starting Date	21-JAN-2004
Experimental Completion Date	04-FEB-2004
Delivery of Animals	21-JAN-2004
Acclimatization	21-JAN-2004 to 27-JAN-2004
Treatment (epicutaneous)	28-JAN-2004 to 30-JAN-2004
Treatment (intravenous)	02-FEB-2004
Observation	21-JAN-2004 to 02-FEB-2004
³ HTdR Determination	03-FEB-2004
Termination	04-FEB-2004

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1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data, sample of test items and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's consent. The remaining test item will be returned to the sponsor. Archiving of the test article is the responsibility of the Sponsor.

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[REDACTED]

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1.5 SIGNATURES

Study Director:

Dr. W. Wang-Fan

W. Wang-Fan
date: 02 April 2004

Test Facility Management:

Dr. H. Fankhauser

H. Fankhauser
date: 02 April 2004

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433
[REDACTED]

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC Study Number 851904
Test Item [REDACTED]
Study Director Dr. W. Wang-Fan
Title [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the RCC Quality Assurance Unit. The dates are given below:

Dates and Types of QAU Inspections		Dates of Reports to the Study Director and to Management
12-DEC-2003	Study Plan	12-DEC-2003
15-JAN-2004	Process Based (Raw Data, Test System, Test Item, Administration,)	15-JAN-2004
25-FEB-2004	Report	25-FEB-2004

This statement also confirms that this final report reflects the raw data.

In addition this final report includes a QAU-Statement issued by the Test Site Quality Assurance Unit.

Lead Quality Assurance:

S. van Dongen

S. van Dongen
date: 2-17-2004

RCC STUDY NUMBER 851904
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[REDACTED]

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GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC Study Number 851904
Test Item [REDACTED]
Study Director Dr. W. Wang-Fan
Title [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The supporting data for purity (characterisation) of the test item was not made available at the time of issuing this report and hence this information has been excluded from the Statement of Compliance. However, the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97) 186/Final].

Study Director:

Dr. W. Wang-Fan

W. Wang - Fan

date: 02 April 2004

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433

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1.8 TEST GUIDELINE

The study procedures described in this report meet or exceed the requirements of the following guideline:

OECD Guideline for the Testing of Chemicals, Guideline 429: Skin Sensitization: Local Lymph Node Assay (adopted 24 April 2002).

The study procedures were optimised to conform with the American regulatory preferences for the local lymph node assay.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 114.

1.10 REFERENCES

Kimber I., Hilton J. and Weisenberger C. (1989). The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. *Contact Dermatitis*, 21, 215-220.

Kimber I. and Basketter D.A. (1992). The murine local lymph node assay. A commentary on collaborative studies and new directions. *Food and Chemical Toxicology*, 30, 165-169.

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Basketter D.A., Lea L.J., Cooper K., Stocks J., Dickens A., Pate I., Dearman R.J. and Kimber I. (1999). Threshold for Classification as a Skin Sensitizer in the Local Lymph Node Assay: A Statistical Evaluation. *Food and Chemical Toxicology*, 37, 1-8.

Steiling W., Basketter D.A., Berthold K., Butler M., Garrigue J-L., Kimber I., Lea L.J., Newsome C., Roggeband R., Stropp G., Waterman S. and Wiemann C. (2001). Skin Sensitisation Testing - New Perspectives and Recommendations. *Food and Chemical Toxicology*, 39, 293-301.

Basketter D.A., Lea L.J., Cooper K., Stocks J., Dickens A., Pate I., Dearman R.J. and Kimber I. (1999). Threshold for Classification as a Skin Sensitiser in the Local Lymph Node Assay: a Statistical Evaluation. *Food and Chemical Toxicology*, 37, 1167-1174.

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2 SUMMARY

In order to study a possible contact allergenic potential of [REDACTED] three groups each of five female mice were treated daily with the test item at concentrations of 5 %, 10 % and 25 % (w/v) in N,N-dimethylformamide (DMF) by topical application to the dorsum of each ear lobe (left and right). A positive control group of five mice was treated with 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v). Two different vehicles were used in this study, therefore, two control groups, each of five mice, were treated with one or other of the two vehicle materials only. It was intended that each of the test and control items would be applied for three consecutive days, however, due to the death of a number of the animals this was not the case for all treatment groups (as detailed below). Five days after the first topical application the surviving mice were injected intravenously into a tail vein with radio-labelled thymidine (³H-methyl thymidine). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per mouse. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were washed subsequently and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ³H-methyl thymidine measured in a β -scintillation counter.

The animal No. 29 (Group 6, 25 %) died one day after the first topical application. The mice Nos. 18 (Group 4, 5 %), 26, 27, 28 and 30 (Group 6, 25 %) died one hour after the second application. The mice Nos. 21 and 23 (Group 5, 10 %) died two hours after the second application. The mice Nos. 22, 24 and 25 (Group 5, 10 %) were euthanized due to severe sedation. The animal No. 20 (Group 4, 5 %) died 20 minutes after the third application. None of the mice in the positive or negative control groups died. All animals which died before the end of the study were dissected by the pathology department and no unusual findings were observed.

No clinical signs were observed in any animals of the two vehicle control groups. On the second application day, a slight ear swelling was observed at both dosing sites in all mice of the positive control Group 3 (25 % HCA), persisting for the remainder of the in-life phase of the study. In addition, a slight ear erythema was observed at both dosing sites in all mice of this group on the third application day, persisting for a total of three days. Approximately two hours after the third application, the three remaining mice of Group 4 (5 %) showed somnolence and decreased spontaneous activity.

The results obtained (STIMULATION INDEX (S.I.)) are reported in the following table.

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Group	%(w/v)	DPM/mouse M ± SD	S.I. (SD)	Statistical Analysis ^{a)} t-test (G = 2, N = 10, t = 2.31) ^{b)} t-test (G = 2, N = 8, t = 2.45)	
				t value	Conclusion
NCG 1	-	875 ± 308	-	-	
NCG 2	-	1077 ± 173	-	-	
PCG 3	25 (HCA)	7239 ± 3829	6.7 (3.6)	3.59 ^{a)}	**
TG 4	5	2617 ± 1623	3.0 (1.9)	2.46 ^{b)}	**

** significant difference at $p \leq 0.05$ (two sides)

NCG 1 Vehicle group = N,N-dimethylformamide (DMF)

NCG 2 Vehicle group = acetone:olive oil, 4:1 (v/v)

PCG 3 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)

TG 4 Test item in N,N-dimethylformamide (DMF)

3 CONCLUSION

A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration of the test item resulted in an incorporation of ³HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the STIMULATION INDEX (S.I.).

In this study a STIMULATION INDEX of 6.7 was obtained with the positive control item ALPHA-HEXYLCINNAMALDEHYDE (HCA) at a concentration of 25% (w/v) in acetone:olive oil, 4:1 (v/v). This S.I. confirms that HCA is a skin sensitizer and is consistent with historical values for HCA at this test concentration. In the t-test a significant difference in the DPM/mouse values was obtained between the positive control (25% ALPHA-HEXYLCINNAMALDEHYDE) group and the vehicle control group at $p \leq 0.05$ (two sides) which also confirms that HCA is a skin sensitizer. The positive control data therefore shows that the assay is consistent and reliable, and produces responses within the expected parameters.

Unfortunately only three of the mice treated with the test item (at the lowest dose level of 5% (w/v)) survived to the end of the study. In the light of this, the variable effects seen in these surviving mice cannot be attributed with confidence either to sensitisation or any other toxicity-related effect. It is therefore impossible to derive a firm conclusion about the sensitisation potential of the test item.

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[REDACTED]

REPORT

4 PURPOSE

The purpose of this Local Lymph Node Assay was to identify the contact allergenic potential of [REDACTED] when administered to the dorsum of both ear lobes of mice.

This study should provide a rational basis for risk assessment to the sensitizing potential of the test item in man.

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[REDACTED]

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5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Mice, CBA/CaOlaHsd
Rationale	Recognized as the recommended test system.
Source	Harlan Netherlands B.V. Postbus 6174 NL - 5960 AD Horst / The Netherlands
Number of animals for the main study	30 females
Number of animals per group	5 females (nulliparous and non-pregnant)
Number of test groups	3
Number of vehicle control groups	2
Number of positive control group	1
Age	8 - 12 weeks (beginning of acclimatization)
Body weight	16 g - 24 g (ordered)
Identification	Each cage by unique cage card.
Randomization	Randomly selected by computer algorithm at time of delivery.
Acclimatization	Under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

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5.2 ALLOCATION

The animals were distributed as follows:

GROUP	CONCENTRATION % (w/v)	NUMBER OF ANIMALS PER GROUP	CAGE NUMBER (Individually housed)
1 Vehicle Control Group ^{a)}	-	5	1 - 5
2 Vehicle Control Group ^{b)}	-	5	6 - 10
3 Positive Control Group ^{c)}	25	5	11 - 15
4 Test Item Group ^{d)}	5	5	16 - 20
5	10	5	21 - 25
6	25	5	26 - 30

^{a)} Vehicle group = N,N-dimethylformamide (DMF)

^{b)} Vehicle group = acetone:olive oil, 4:1 (v/v)

^{c)} 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)

^{d)} Test item in N,N-dimethylformamide (DMF)

5.3 HUSBANDRY

Room no.

E21 / RCC Ittingen

Conditions

Standard Laboratory Conditions. Air-conditioned with target ranges for room temperature 22 ± 3 °C, relative humidity 30 - 70 % and 10 - 15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. There was a 12 hour fluorescent light / 12 hour dark cycle with at least 8 hours music during the light period.

Accommodation

Individual in Makrolon type-2 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 MuttENZ).

Diet

Pelleted standard Kliba 3433, batch no. 78/03 mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst) available *ad libitum*. Results of analyses for contaminants are archived at RCC.

Water

Community tap water from Ittingen, available *ad libitum*. Results of representative bacteriological, chemical and contaminant analyses are archived at RCC.

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5.4 CHEMICALS

³H-methyl Thymidine

Amersham TRA 310, batch no. 313 aqueous solution, sterilized 74 GBq/mmol (2 Ci/mmol), 37 MBq/ml (1 mCi/ml) quantities: 9.25 MBq (250 µCi), 37 MBq (1 mCi)

Trichloroacetic acid

Fluka no. 91230 (min. 99.5 %)

Phosphate buffered saline

Fluka no. 79382 (1 tablet solved in 200 ml bi-distilled water)

5.5 VEHICLES

N,N-Dimethylformamide (DMF)

Supplier

Merck KGaA (Frankfurter Str. 250, D-64293 Darmstadt, Germany)

Batch number

1.02937.0500

Expiry date

31-MAY-2006

Storage conditions

In the original container at room temperature (20 °C ± 3 °C), away from direct sunlight

Acetone:olive oil, 4:1 (v/v)

1) Acetone

Supplier

Baker, P. H. Stehelin & Cie AG (Spalentorweg 62, CH-4003 Basel, Switzerland)

Batch number

0310810002

Expiry date

AUG-2004

Storage conditions

In the original container at room temperature (20 °C ± 3 °C), away from direct sunlight.

2) Olive oil

Supplier

Roth AG (Chr. Merian-Ring 7, CH-4153 Reinach BL, Switzerland)

Batch number

22357895

Expiry date

09-SEP-2004

Storage conditions

In the original container at room temperature (20 °C ± 3 °C), away from direct sunlight.

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[REDACTED]

REPORT

5.6 TEST ITEM

Identity	[REDACTED]
Description	[REDACTED]
[REDACTED] Sample number	S2539801
Stability of test item	Stable under storage conditions
Expiry date	01-JAN-2005
Storage conditions	In the original container at room temperature (20 °C ± 3 °C). Keep in dark.
Safety precautions	Routine hygienic procedures (gloves, goggles, face mask).

The supporting data for purity (characterisation) of the test item was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However the sponsor has addressed this in a GLP compliant study, [REDACTED] Study Reference No. AC030449.

The test item information was supplied by the Sponsor.

5.6.1 POSITIVE CONTROL ITEM

Identity	, ALPHA-HEXYLCINNAMALDEHYDE
Description	liquid
Batch number	13102MO
Purity	tech., 85 %
Stability of test item	Stable under storage conditions
Expiry date	08-DEC-2005
Storage conditions	In the original container at room temperature (20 °C ± 3 °C), away from direct sunlight.
Safety precautions	Routine hygienic procedures (gloves, goggles, face mask).

This information was supplied by the supplier.

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5.7 TEST ITEM FORMULATIONS PREPARATION

The test item and the positive control item ALPHA-HEXYLCINNAMALDEHYDE were placed into a volumetric flask on a tared Mettler balance, and vehicles N,N-dimethylformamide (DMF) or acetone:olive oil, 4:1 (v/v), respectively, were quantitatively added separately. The weight/volume dilutions were prepared individually.

Test item and positive control item formulations were made freshly before each dosing occasion and no more than 4 hours prior to application to the ears.

Homogeneity of the test item and positive control item in vehicles was maintained during treatment by use of a magnetic stirrer.

The test item was assayed at three consecutive concentrations selected by the Sponsor.

Concentrations were in terms of material as supplied.

5.8 RATIONALE

The study procedure was used to detect a possible contact allergenic potential of the test item applied.

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433

REPORT

6 STUDY CONDUCT

6.1 TREATMENT PROCEDURES

6.1.1 TOPICAL APPLICATION

Each test group of mice was treated by (epidermal) topical application to the dorsal surface of each ear lobe (left and right) with the test item at different concentrations. A further three groups of mice were treated with an equal volume of either, the positive control item dilution, the positive control vehicle (AOO) or the negative control material (DMF). The application volume, 25ul, was spread over the entire dorsal surface ($\varnothing \sim 8$ mm) of each ear lobe. A hair dryer was used to dry the ear's surface as quickly as possible to avoid loss of test item applied. It was intended that each of the test and control items would be applied once daily for 3 consecutive days, however, due to the death of a number of the animals this was not the case for all treatment groups (as detailed in section 7.2).

6.1.2 ADMINISTRATION OF ^3H -METHYL THYMIDINE*

^3H -methyl thymidine ($^3\text{HTdR}$) was purchased from Amersham International (Amersham product code no. TRA 310; specific activity, 2 Ci/mmol; concentration, 1 mCi/ml).

Five days after the first topical application, all surviving mice were administered with 250 μl of 86.5 $\mu\text{Ci/ml}$ $^3\text{HTdR}$ (equal to 21.6 μCi $^3\text{HTdR}$) by intravenous injection via a tail vein.

6.1.3 DETERMINATION OF INCORPORATED $^3\text{HTdR}$ *

Approximately five hours after treatment with $^3\text{HTdR}$ all surviving mice were euthanized by intraperitoneal injection of VETANARCOL (Veterinaria AG, Zürich).

The draining lymph nodes were rapidly excised and pooled for each individual animal (2 nodes per mouse). Single cell suspensions (phosphate buffered saline) of pooled lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze (200 μm mesh size). After washing two times with phosphate buffered saline (approx. 10 ml) the lymph node cells were resuspended in 5 % trichloroacetic acid (approx. 3 ml) and incubated at approximately +4 °C for at least 18 hours for precipitation of macromolecules. The precipitates were then resuspended in 5 % trichloroacetic acid (1 ml) and transferred to glass scintillation vials with 10 ml of 'Ultima Gold' scintillation liquid and thoroughly mixed.

The level of $^3\text{HTdR}$ incorporation was then measured on a β -scintillation counter. Similarly, background $^3\text{HTdR}$ levels were also measured in two 1ml-aliquots of 5 % trichloroacetic acid. The β -scintillation counter expresses $^3\text{HTdR}$ incorporation as the number of radioactive disintegrations per minute (DPM).

* Preparation of $^3\text{HTdR}$ solutions and $^3\text{HTdR}$ measurements at RCC Ltd, Environmental Chemistry & Pharamanalytics

No phase report of the results of the $^3\text{HTdR}$ level analysis was provided by the Principal Investigator.

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6.1.4 INTERPRETATION OF RAW DATA

The proliferative responses of lymph node cells are expressed as the number of radioactive disintegrations per minute per animal (DPM/mouse). The mean DPM/mouse value was calculated for each of the test and control groups that survived until the end of the study. The ratio of ³HTdR incorporated into lymph node cells of test lymph nodes relative to that recorded for the relevant vehicle control lymph nodes (STIMULATION INDEX) was calculated by dividing the mean DPM/mouse for each test group by the mean DPM/mouse of the relevant vehicle control group. Before DPM/mouse values are determined, mean scintillation-background DPM will be subtracted from test and control raw data.

A test item is regarded as a sensitizer in the LLNA if the following criteria are fulfilled:

- First, that exposure to at least one concentration of the test item resulted in an incorporation of ³HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the STIMULATION INDEX (S.I.).
- Second, that the data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

6.2 OBSERVATIONS

In addition to the sensitizing reactions the following observations and data were recorded during the test and observation period:

Mortality / Viability	Twice daily from acclimatization start to the termination of in-life phase.
Body weights	Prior to the 1 st application and prior to necropsy.
Clinical signs (local / systemic)	Daily from acclimatization start to the termination of in-life phase. Particular attention was paid to the treatment sites.

6.3 STATISTICAL ANALYSIS

The mean body weights and mean DPM/mouse values for each test and control group (that survived until the end of the study) were calculated. Standard deviations of the data used to determine these mean values were calculated.

The t-test was conducted for the assessment of significant differences between the positive control item group and its vehicle control group, and between the test item group and its vehicle control group, separately. Study plan amendment 2 stated that a dunnett-test would be used to assess the significant differences between the test item groups and the vehicle control group, however because only one test item group survived until the end of the study a t-test was sufficient and a dunnett-test was not conducted.

6.4 DATA COMPILATION

Body weights will be recorded on-line in RCC-TOX LIMS.

Clinical signs were compiled directly into the RCC computer system.

Mortality/viability were compiled on data sheets.

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Sponsor's Reference Number KSL030433

REPORT

7 RESULTS

7.1 CALCULATION AND RESULTS OF INDIVIDUAL DATA

The proliferative capacity of pooled lymph node cells was determined by the incorporation of ³H-methyl thymidine measured on a β -scintillation counter. The values measured are given in Appendix A.

Group	% (w/v)	DPM/mouse M \pm SD	S.I. (SD)	Statistical Analysis ^{a)} t-test (G = 2, N = 10, t = 2.31) ^{b)} t-test (G = 2, N = 8, t = 2.45)	
				t value	Conclusion
NCG 1	-	875 \pm 308	-	-	
NCG 2	-	1077 \pm 173	-	-	
PCG 3	25 (HCA)	7239 \pm 3829	6.7 (3.6)	3.59 ^{a)}	**
TG 4	5	2617 \pm 1623	3.0 (1.9)	2.46 ^{b)}	**

** significant difference at $p \leq 0.05$ (two sides)

NCG 1 Vehicle group = N,N-dimethylformamide (DMF)

NCG 2 Vehicle group = acetone:olive oil, 4:1 (v/v)

PCG 3 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)

TG 4 Test item in N,N-dimethylformamide (DMF)

The radioactive disintegration values for the individual treatment animals are included in Appendix A.

7.2 VIABILITY / MORTALITY

The animal No. 29 (Group 6, 25 %) died one day after the first topical application. The mice Nos. 18 (Group 4, 5 %), 26, 27, 28 and 30 (Group 6, 25 %) died one hour after the second application. The mice Nos. 21 and 23 (Group 5, 10 %) died two hours after the second application. The mice Nos. 22, 24 and 25 (Group 5, 10 %) were euthanized due to severe sedation. The animal No. 20 (Group 4, 5 %) died 20 minutes after the third application. None of the mice in the positive or negative control groups died. All animals which died before the end of the study were dissected by the pathology department and no unusual findings were observed.

7.3 CLINICAL SIGNS

No clinical signs were observed in any animals of the two vehicle control groups. On the second application day, a slight ear swelling was observed at both dosing sites in all mice of the positive control Group 2 (25 % HCA), persisting for the remainder of the in-life phase of the study. In addition, a slight ear erythema was observed at both dosing sites in all mice of

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this group on the third application day, persisting for a total of three days. Approximately two hours after the third application, the three remaining mice of Group 4 (5 %) showed somnolence and decreased spontaneous activity.

The individual clinical signs are included in Appendix B.

(In Appendix B the numbers in brackets, e.g. (4) show that the severity of the symptoms may be classified into four grades: slight (1), moderate (2), severe (3) and very severe (4); the points indicate the application days; the numbers indicate the severity of the symptoms.)

7.4 BODY WEIGHTS

The body weights of the animals, recorded prior to the 1st application (for all animals) and prior to necropsy (for all surviving mice), was within the range commonly recorded for animals of this strain and age.

Animals which did not survive until the end of the study were not weighed again because carcasses dry out and loose weight, meaning that the final weight would not have been accurate.

The individual as well as groupwise summarised body weight values are included in Appendix C.

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[REDACTED]

REPORT

APPENDIX A

CALCULATION AND RESULTS OF INDIVIDUAL DATA

RCC Study Number 851904

Sponsor's Reference Number KSL030433

Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens)

The following results were obtained:

Vehicles: (1) N,N-dimethylformamide (DMF) (2) acetone:olive oil, 4:1 (v/v)

No.	Test Item	% w/v	Group	dpm - BG ^{a)}	Ln N	dpm/Mouse	Dpm/Mouse M (SD)	Statistical Analyses	Statistical Significance	S.I.	S.I. M	S.I. SD
--	--	--	BG I	3	--	--	--			--	--	--
--	--	--	BG II	6	--	--	--			--	--	--
1	--	--	NCG1	861	856	2	856	875		--	--	--
2	--	--	NCG1	936	931	2	931	(308)		--	--	--
3	--	--	NCG1	647	642	2	642			--	--	--
4	--	--	NCG1	589	584	2	584			--	--	--
5	--	--	NCG1	1366	1361	2	1361			--	--	--
6	--	--	NCG2	1357	1352	2	1352	1077		--	--	--
7	--	--	NCG2	1143	1138	2	1138	(173)		--	--	--
8	--	--	NCG2	990	985	2	985			--	--	--
9	--	--	NCG2	924	919	2	919			--	--	--
10	--	--	NCG2	995	990	2	990			--	--	--
11	HCA	25	PCG3	3723	3718	2	3718	7239	t-test	3.5		
12	HCA	25	PCG3	6245	6240	2	6240	(3829)	(G = 2, N = 10, t = 2.31)	5.8		
13	HCA	25	PCG3	6235	6230	2	6230		t = 3.59	5.8	6.7	3.6
14	HCA	25	PCG3	6205	6200	2	6200			5.8		
15	HCA	25	PCG3	13813	13808	2	13808			12.8		
16	TI	5	TG4	1058	1053	2	1053	2617	t-test	1.2		
17	TI	5	TG4	2509	2504	2	2504	(1623)	(G = 2, N = 8, t = 2.45)	2.9	3.0	1.9
19	TI	5	TG4	4298	4293	2	4293		t = 2.46	4.9		

* significant difference at $p \leq 0.05$ (two sides)

NCG1 = N,N-dimethylformamide (DMF)

NCG2 = acetone:olive oil, 4:1 (v/v)

PCG3 = 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE (HCA) in acetone:olive oil, 4:1 (v/v)

TG4 = Test item in N,N-dimethylformamide (DMF)

BG = Background (1 ml 5 % trichloroacetic acid) in duplicate

M = Mean

SD = Standard Deviation

S.I. = Stimulation Index

^a = The mean value was taken from the figures BG I and BG II

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[REDACTED]

REPORT

APPENDIX B

INDIVIDUAL / SUMMARY CLINICAL SIGNS

Report

RCC STUDY NUMBER 851904
[REDACTED]

SYM-IND - 1

04-FEB-04

CLINICAL SIGNS, DAILY
FEMALES
GROUP 1 (NEG. CONTROL GROUP)SIGN (MAX.GRADE)
(LOCATION)ACCLIMATISATION
WEEKS: 1.....TREATMENT
1.....

ANIMAL 1

NO CLINICAL SIGNS NOTED

ANIMAL 2

NO CLINICAL SIGNS NOTED

ANIMAL 3

NO CLINICAL SIGNS NOTED

ANIMAL 4

NO CLINICAL SIGNS NOTED

ANIMAL 5

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-IND - 2
04-FEB-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 2 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 6

NO CLINICAL SIGNS NOTED

ANIMAL 7

NO CLINICAL SIGNS NOTED

ANIMAL 8

NO CLINICAL SIGNS NOTED

ANIMAL 9

NO CLINICAL SIGNS NOTED

ANIMAL 10

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 851904

Report

SYM-IND - 3
04-FEB-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 3 (POS. CONTROL GROUP 25%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
ANIMAL 11		

SKIN / FUR		
SWELLING (3)	G:11111
(EAR LEFT)		
SWELLING (3)	G:11111
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR RIGHT)		
ANIMAL 12		

SKIN / FUR		
SWELLING (3)	G:11111
(EAR LEFT)		
SWELLING (3)	G:11111
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR RIGHT)		
ANIMAL 13		

SKIN / FUR		
SWELLING (3)	G:11111
(EAR LEFT)		
SWELLING (3)	G:11111
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR RIGHT)		
ANIMAL 14		

SKIN / FUR		
SWELLING (3)	G:11111
(EAR LEFT)		
SWELLING (3)	G:11111
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR RIGHT)		
ANIMAL 15		

SKIN / FUR		
SWELLING (3)	G:11111
(EAR LEFT)		
SWELLING (3)	G:11111
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:111.
(EAR RIGHT)		

G: Highest daily grades

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-IND - 4
04-FEB-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 4 (TEST GROUP 5%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
ANIMAL 16		

BEHAVIOR		
SOMNOLENT (1)	G:1...
DECREAS. SPONT. ACTIVITY (3)	G:1...
ANIMAL 17		

BEHAVIOR		
SOMNOLENT (1)	G:1...
DECREAS. SPONT. ACTIVITY (3)	G:1...
ANIMAL 18		

NO CLINICAL SIGNS NOTED		
ANIMAL 19		

BEHAVIOR		
SOMNOLENT (1)	G:1...
DECREAS. SPONT. ACTIVITY (3)	G:1...
ANIMAL 20		

NO CLINICAL SIGNS NOTED		

G: Highest daily grades

Report

RCC STUDY NUMBER 851904
[REDACTED]SYM-IND - 5
04-FEB-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 5 (TEST GROUP 10%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
<hr/>		
ANIMAL 21		

NO CLINICAL SIGNS NOTED		
ANIMAL 22		

NO CLINICAL SIGNS NOTED		
ANIMAL 23		

NO CLINICAL SIGNS NOTED		
ANIMAL 24		

NO CLINICAL SIGNS NOTED		
ANIMAL 25		

NO CLINICAL SIGNS NOTED		

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-IND - 6
04-FEB-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 6 (TEST GROUP 25%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
<hr/>		
ANIMAL 26		

NO CLINICAL SIGNS NOTED		
ANIMAL 27		

NO CLINICAL SIGNS NOTED		
ANIMAL 28		

NO CLINICAL SIGNS NOTED		
ANIMAL 29		

NO CLINICAL SIGNS NOTED		
ANIMAL 30		

NO CLINICAL SIGNS NOTED		

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-SUM - 1
04-FEB-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 1 (NEG. CONTROL GROUP)SIGN (MAX.GRADE)
LOCATIONACCLIMATISATION
WEEKS: 1.....TREATMENT
1.....

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-SUM - 2
04-FEB-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 2 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE)	ACCLIMATISATION	TREATMENT
LOCATION	WEEKS: 1.....	1.....

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-SUM - 3
04-FEB-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 3 (POS. CONTROL GROUP 25%)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
SKIN / FUR		

SWELLING (3) (EAR LEFT)	G: %:11111 .AAAAA
SWELLING (3) (EAR RIGHT)	G: %:11111 .AAAAA
GENERAL ERYTHEMA (4) (EAR LEFT)	G: %:111. ..AAA.
GENERAL ERYTHEMA (4) (EAR RIGHT)	G: %:111. ..AAA.

G: Median value of the highest individual daily grades
%: Percent of affected animals (0 = less than 5%, 1 = between 5% and 15%, ..., A = more than 95%) 34

RCC STUDY NUMBER 851904
[REDACTED]

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SYM-SUM - 4
04-FEB-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 4 (TEST GROUP 5%)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
<hr/>		
BEHAVIOR		

SOMNOLENT (1)	G:1...
	%:8...
DECREAS. SPONT. ACTIVITY (3)	G:1...
	%:8...

G: Median value of the highest individual daily grades

%: Percent of affected animals (0 = less than 5%, 1 = between 5% and 15%, ..., A = more than 95%) 35

RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-SUM - 5
04-FEB-04**CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 5 (TEST GROUP 10%)**

SIGN (MAX.GRADE)	ACCLIMATISATION	TREATMENT
LOCATION	WEEKS: 1.....	1.....

NO CLINICAL SIGNS NOTED

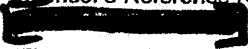
RCC STUDY NUMBER 851904
[REDACTED]

Report

SYM-SUM - 6
04-FEB-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 6 (TEST GROUP 25%)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
------------------------------	----------------------------------	---------------------

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433


REPORT

APPENDIX C

INDIVIDUAL / SUMMARY BODY WEIGHTS

Report

RCC STUDY NUMBER 851904
[REDACTED]BW-IND - 1
04-FEB-04BODY WEIGHTS (GRAM)
FEMALES

	TREATMENT	

DAYS	1	6
WEEKS	1	1
ANIMAL		

GROUP 1 (NEG. CONTROL GROUP)

1	19.5	20.0
2	20.9	21.9
3	21.7	22.1
4	18.6	18.9
5	19.7	20.1

GROUP 2 (NEG. CONTROL GROUP)

6	22.3	23.1
7	20.9	20.8
8	20.9	20.8
9	21.6	21.6
10	22.2	21.6

GROUP 3 (POS. CONTROL GROUP 25%)

11	21.3	22.5
12	23.3	24.7
13	18.3	19.1
14	20.7	21.9
15	21.0	23.0

GROUP 4 (TEST GROUP 5%)

16	21.2	23.3
17	20.2	20.0
18	21.2	---
19	18.4	19.2
20	21.1	---

GROUP 5 (TEST GROUP 10%)

21	21.1	---
22	21.5	---
23	20.4	---
24	22.0	---
25	20.2	---

GROUP 6 (TEST GROUP 25%)

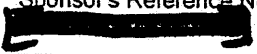
26	21.7	---
27	19.8	---
28	21.9	---
29	21.3	---
30	21.0	---

RCC STUDY NUMBER 851904

Report

BW-SUM - 1
04-FEB-04BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 NEG. CONTROL GROUP	GROUP 2 NEG. CONTROL GROUP	GROUP 3 POS. CONTROL GROUP 25%
DAY 1	MEAN	20.1	21.6	20.9
WEEK 1	ST.DEV.	1.2	0.7	1.8
	N	5	5	5
		GROUP 4 TEST GROUP 5%	GROUP 5 TEST GROUP 10%	GROUP 6 TEST GROUP 25%
	MEAN	20.4	21.0	21.2
	ST.DEV.	1.2	0.8	0.8
	N	5	5	5
		GROUP 1 NEG. CONTROL GROUP	GROUP 2 NEG. CONTROL GROUP	GROUP 3 POS. CONTROL GROUP 25%
DAY 6	MEAN	20.6	21.6	22.2
WEEK 1	ST.DEV.	1.4	0.9	2.0
	N	5	5	5
		GROUP 4 TEST GROUP 5%	GROUP 5 TEST GROUP 10%	GROUP 6 TEST GROUP 25%
	MEAN	20.8	---	---
	ST.DEV.	2.2	---	---
	N	3	0	0

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433


REPORT

APPENDIX D

GOOD LABORATORY PRACTICE

- STATEMENT OF COMPLIANCE (PRINCIPAL INVESTIGATOR)**
- QUALITY ASSURANCE UNIT (PRINCIPAL INVESTIGATOR)**

RCC STUDY NUMBER 851904

REPORT

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

RCC Study Number: 851904

Study Director: Dr. W. Wang-Fan, Toxicology

Test Item: [REDACTED]

Principal Investigator
³HTdR Determination: Dr. R. Burri, Environmental Chemistry &
Pharmanalytics

Phase to: [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The preparation of the [methyl-³H]Thymidine solution and determination of radioactivity content were conducted in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Principal Investigator
³HTdR Determination:

Dr. R. Burri

R. Burri
.....
Date:

February 12, 2004

RCC STUDY NUMBER 851904

REPORT

QUALITY ASSURANCE UNIT

RCC Ltd, Environmental Chemistry & Pharamanalytics, CH-4452 Itingen / Switzerland

STATEMENT

RCC Study Number: 851904

Study Director: Dr. W. Wang-Fan, Toxicology

Test Item:

Principal Investigator
³HTdR Determination:

Dr. R. Burri, Environmental Chemistry &
Pharmanalytics

Phase to:

Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected by the quality assurance. The date is given below.

Dates and Types of QA Inspections		Dates of Reports to the Principal Investigator and to the Management
January 16, 2004	Process based (preparation of application solution)	January 16, 2004

Sections of the draft study plan relating to the phase were reviewed and reported to the study director, lead QA and test facility management on December 11, 2003

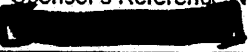
Summary report(s) of study related inspection(s) (if applicable) were issued to the study director, lead QA and test facility management.

Quality Assurance:

Mr. Jürgen Lütte

Date:

February 12, 2004

RCC STUDY NUMBER 851904
Sponsor's Reference Number KSL030433


REPORT

APPENDIX E

GLP - CERTIFICATION

RCC STUDY NUMBER 851904

REPORT

Sponsor's Reference Number KSL030433

The Swiss GLP Monitoring Authorities

Swiss Federal
Office of
Public HealthSwiss Agency for the
Environment, Forests
and LandscapeIntercantonal Office
for the Control of
Medicines

Statement of GLP Compliance

It is hereby confirmed that

during the period of

August 15 – 17, 2000

August 28 - 29, 2001 and

April 15, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for the Environment, Forests and Landscape and the Intercantonal Office for the Control of Medicines with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities**areas of expertise*****- Toxicology Division****TOX, ACC, MUT****- Environmental Chemistry and
Pharmanalytics Division****ACC, ECT, ENF, EMN,
PCT, RES, OTH (Animal
metabolism)****- Microbiological Diagnostics by
Biotechnology & Animal Breeding Division****OTH (Microbiology)**

The inspection was performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Prof. Th. Zeltner

Bern, May 2002

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air, Bioaccumulation ; EMN = Studies on effects on mesocosms and natural ecosystems ; MUT = Mutagenicity ; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.

RCC Study Number 854128

Sponsor's Reference Number KSL040164

[REDACTED]

Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens)

Report Amendment No.1


Author:

Dr. W. Wang-Fan

Sponsor:

[REDACTED]

Page 1 of 4
Total Number of Pages: 4

RCC STUDY NUMBER 854128
SPONSOR'S REFERENCE NUMBER KSL040164


REPORT AMENDMENT NO. 1

Page 2

SIGNATURES

STUDY DIRECTOR:

Dr. W. Wang-Fan

W Wang-Fan
date: 30 July 2004

TEST FACILITY MANAGEMENT:

Dr. H. Fankhäuser

H. Fankhäuser
date: 30 July 2004

RCC STUDY NUMBER 854128
SPONSOR'S REFERENCE NUMBER KSL040164
[REDACTED]

REPORT AMENDMENT NO. 1

Page 3

LEAD QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC Study Number

854128

Test Item

[REDACTED]

Study Director

Dr. W. Wang-Fan

Title

[REDACTED]

Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

This Amendment to the Report was audited by the RCC Quality Assurance Unit. The date is given below.

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Dates and Types of QAU Inspections		Dates of Reports to the Study Director and to Management
30-JUL-2004	Amendment No.1 to Report	30-JUL-2004

This statement also confirms that this Amendment to Report reflects the raw data.

Lead Quality Assurance:

S. van Dongen

S. van Dongen
.....
date: 30 - Jul - 2004

RCC STUDY NUMBER 854128
SPONSOR'S REFERENCE NUMBER KSL040164

REPORT AMENDMENT NO. 1

Page 4

PAGE

23

CONCERNING

CALCULATION AND RESULTS OF INDIVIDUAL DATA

PRESENT

dpm/LN

NEW

dpm/Mouse

REASON FOR THE ALTERATION

Typing error.

DISTRIBUTION

This amendment to report will be distributed as follows:

Sponsor:

1 Copy Scientific Representative (responsible for distribution to the Sponsor company)

RCC Ltd, TOX, Itingen:

Original Study File

1 Copy Lead QA

RCC Ltd, Environmental Chemistry & Pharmanalytics, Itingen:

1 Copy Test Site QA

RCC Study Number 854128

Sponsor's Reference Number KSL040164

[REDACTED]

Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens)

Report

Author:

Dr. W. Wang-Fan

Sponsor:

[REDACTED]

Study Completion Date: 26 July 2004

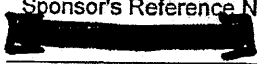
Total Number of Pages: 45

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

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RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

1 PREFACE

1.1 GENERAL

Title

[REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

Sponsor

Scientific Representative

[REDACTED] Contacts

Test Facility

a) RCC Ltd
Toxicology
CH - 4452 Itingen / Switzerland

Test Site

b) RCC Ltd
Environmental Chemistry & Pharamanalytics
CH - 4452 Itingen / Switzerland

Lead QA

RCC Ltd
Quality Assurance GLP
Toxicology
CH - 4452 Itingen / Switzerland

Test Site QA

RCC Ltd
Quality Assurance GLP
Environmental Chemistry & Pharamanalytics
CH - 4452 Itingen / Switzerland
(Responsible for test site)

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

1.2 RESPONSIBILITIES

Study Director	Dr. W. Wang-Fan (a)
Deputy Study Director	L.G. Ullmann (a)
Technical Coordinator / Necropsy	N. Schäfer (a)
Head of Lead Quality Assurance	I. Wüthrich

Principal Investigator

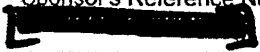
Study Phase: ³ HTdR Determination	Dr. R. Burri (b)
--	------------------

1.3 SCHEDULE

Experimental Starting Date	05-MAY-2004
Experimental Completion Date	19-MAY-2004
Delivery of Animals	05-MAY-2004
Acclimatization	05-MAY-2004 to 11-MAY-2004
Treatment (epicutaneous)	12-MAY-2004 to 14-MAY-2004
Treatment (intravenous)	17-MAY-2004
Observation	05-MAY-2004 to 17-MAY-2004
³ HTdR Determination	18-MAY-2004

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data of the test facility and the test site, sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's consent. The remaining test item will be returned to the sponsor. Archiving of the test item is the responsibility of the Sponsor.

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164


REPORT

1.5 SIGNATURES

Study Director:

Dr. W. Wang-Fan

W. Wang-Fan

date: 26 July 2004

Test Facility Management:

Dr. H. Fankhauser

H. Fankhauser

date: 26 July 2004

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Ittingen / Switzerland

STATEMENT

RCC Study Number

854128

Test Item

[REDACTED]

Study Director

Dr. W. Wang-Fan

Title

[REDACTED]

Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the RCC Quality Assurance Unit. The dates are given below:

Dates and Types of QAU Inspections		Dates of Reports to the Study Director and to Management
30-APR-2004	Study Plan	30-APR-2004
06-MAY-2004	Process Based (Raw Data, Test System, Test Item, Administration, Observation)	06-MAY-2004
08-JUL-2004	Report	08-JUL-2004

This statement also confirms that this final report reflects the raw data.

In addition this final report includes a QAU-Statement issued by the Test Site Quality Assurance Unit.

Lead Quality Assurance:

S. van Dongen

S. van Dongen
date: 26-Jul-2004

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC Study Number 854128
Test Item [REDACTED]
Study Director Dr. W. Wang-Fan
Title [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The purity (characterisation), stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information was excluded from the statement of compliance. However the sponsor was addressing this in a GLP compliant study [REDACTED] Study Reference No. AC030449.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97) 186/Final].

Study Director:

Dr. W. Wang-Fan

W. Wang - Fan
date: 26 July 2004

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

1.8 TEST GUIDELINE

The study procedures described in this report meet or exceed the requirements of the following guideline:

OECD Guideline for the Testing of Chemicals, Guideline 429: Skin Sensitization: Local Lymph Node Assay (adopted 24 April 2002).

The study procedures were optimised to conform with the American regulatory preferences for the local lymph node assay.

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 114.

1.10 REFERENCES

Kimber I., Hilton J. and Weisenberger C. (1989). The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. *Contact Dermatitis*, 21, 215-220.

Kimber I. and Basketter D.A. (1992). The murine local lymph node assay. A commentary on collaborative studies and new directions. *Food and Chemical Toxicology*, 30, 165-169.

Basketter D.A., Gerbrick G.F., Kimber I. and Loveless S.E. (1996). The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. *Food and Chemical Toxicology*, 34, 985-997.

Chamberlain M. and Basketter D.A. (1996). The local lymph node assay: status of validation. *Food and Chemical Toxicology*, 34, 999-1002.

Basketter D.A., Lea L.J., Cooper K., Stocks J., Dickens A., Pate I., Dearman R.J. and Kimber I. (1999). Threshold for Classification as a Skin Sensitizer in the Local Lymph Node Assay: A Statistical Evaluation. *Food and Chemical Toxicology*, 37, 1-8.

Stelling W., Basketter D.A., Berthold K., Butler M., Garrigue J-L., Kimber I., Lea L.J., Newsome C., Roggeband R., Stropp G., Waterman S. and Wiemann C. (2001). Skin Sensitisation Testing - New Perspectives and Recommendations. *Food and Chemical Toxicology*, 39, 293-301.

Basketter D.A., Lea L.J., Cooper K., Stocks J., Dickens A., Pate I., Dearman R.J. and Kimber I. (1999). Threshold for Classification as a Skin Sensitiser in the Local Lymph Node Assay: a Statistical Evaluation. *Food and Chemical Toxicology*, 37, 1167-1174.

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

2 SUMMARY

In order to study a possible contact allergenic potential of [REDACTED] three groups each of five female mice were treated daily with the test item at concentrations of 0.25 %, 0.5 % and 1 % (w/v) in N,N-dimethylformamide (DMF) by topical application to the dorsum of each ear lobe (left and right) for three consecutive days. A positive control group of five mice was treated with 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v). Two different vehicles were used in this study, therefore, two control groups, each of five mice, were treated with one or other of the two vehicle materials only. Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (³H-methyl thymidine). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per mouse. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were washed subsequently and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ³H-methyl thymidine measured in a β -scintillation counter.

All treated animals survived the scheduled study period.

No clinical signs were observed in any animals of the two vehicle control groups (Groups 1-2) or the three test item groups (Groups 4-6). On the second application day, a slight ear erythema was observed at both dosing sites in all mice of the positive control Group 3 (25 % HCA), persisting for a total of three days. In addition, on the third application day, a slight ear swelling was observed at both dosing sites in all mice of this group, persisting for a total of two days.

The results obtained (STIMULATION INDEX (S.I.)) are reported in the following table.

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Group	% (w/v)	DPM/mouse M ± SD	S.I. (SD)	Statistical Analysis ^{a)} t-test (G = 2, N = 10, t = 2.31) ^{b)} Dunnett-test (G = 4, N = 20, t = 2.59)	
				t value	Conclusion
NCG 1	-	598 ± 135	-	-	--
NCG 2	-	691 ± 190	-	-	--
PCG 3	25 (HCA)	7565 ± 1770	11.0 (2.6)	8.63 ^{a)}	**
TG 4	0.25	2059 ± 280	3.4 (0.5)	4.03 ^{b)}	**
TG 5	0.5	2324 ± 803	3.9 (1.3)	4.76 ^{b)}	**
TG 6	1	2691 ± 758	4.5 (1.3)	5.77 ^{b)}	**
** significant difference at p ≤ 0.05 (two sides)					
-- no significant difference at p ≤ 0.05 (two sides)					
NCG 1	Vehicle group = N,N-dimethylformamide (DMF)				
NCG 2	Vehicle group = acetone:olive oil, 4:1 (v/v)				
PCG 3	25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)				
TG 4-6	Test item in N,N-dimethylformamide (DMF)				
A dose-response relation was observed.					
An EC3 value could not be determined because this calculation requires an S.I. value of less than 3.					

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Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

3 CONCLUSION

A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration of the test item resulted in an incorporation of $^3\text{HTdR}$ at least 3-fold or greater than that recorded in control mice, as indicated by the STIMULATION INDEX (S.I.).

In this study a STIMULATION INDEX of 11.0 was obtained with the positive control item ALPHA-HEXYLCINNAMALDEHYDE (HCA) at a concentration of 25% (w/v) in acetone:olive oil, 4:1 (v/v). This S.I. confirms that HCA is a skin sensitizer. In the t-test a significant difference in the DPM/mouse values was obtained between the positive control (25% ALPHA-HEXYLCINNAMALDEHYDE) group and the vehicle control group at $p \leq 0.05$ (two sides) which also confirms that HCA is a skin sensitizer. The positive control data therefore shows that the assay is consistent and reliable, and produces responses within the expected parameters.

In this study STIMULATION INDICES of 3.4, 3.9 and 4.5 were determined with the test item at concentrations of 0.25 %, 0.5 % and 1 % (w/v) in N,N-dimethylformamide (DMF). Stainless E-700-2003 was therefore found to be a skin sensitizer. An EC3 value could not be determined because this calculation requires an S.I. value of less than 3.

In the Dunnett-test a significant difference in the DPM/mouse values was obtained between each test item group and the vehicle control group at $p \leq 0.05$ (two sides) which also confirms that the test item [REDACTED] is a skin sensitizer.

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[REDACTED]

REPORT

4 PURPOSE

The purpose of this Local Lymph Node Assay was to identify the contact allergenic potential of [REDACTED] when administered to the dorsum of both ear lobes of mice.

This study should provide a rational basis for risk assessment to the sensitizing potential of the test item in man.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Mice, CBA/CaOlaHsd
Rationale	Recognized as the recommended test system.
Source	Harlan Netherlands B.V. Postbus 6174 NL - 5960 AD Horst / The Netherlands
Number of animals for the main study	30 females
Number of animals per group	5 females (nulliparous and non-pregnant)
Number of test groups	3
Number of vehicle control groups	2
Number of positive control group	1
Age	8 - 12 weeks (beginning of acclimatization)
Body weight	16 g - 24 g (ordered)
Identification	Each cage by unique cage card.
Randomization	Randomly selected by computer algorithm at time of delivery.
Acclimatization	Under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

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5.2 ALLOCATION

The animals were distributed as follows:

GROUP	CONCENTRATION % (w/v)	NUMBER OF ANIMALS PER GROUP	CAGE NUMBER (Individually housed)
1 Vehicle Control Group ^{a)}	-	5	1 - 5
2 Vehicle Control Group ^{b)}	-	5	6 - 10
3 Positive Control Group ^{c)}	25	5	11 - 15
4 Test Item Group ^{d)}	0.25	5	16 - 20
5	0.5	5	21 - 25
6	1	5	26 - 30

^{a)} Vehicle group = N,N-dimethylformamide (DMF)

^{b)} Vehicle group = acetone:olive oil, 4:1 (v/v)

^{c)} 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)

^{d)} Test item in N,N-dimethylformamide (DMF)

5.3 HUSBANDRY

Room no.	129 B / RCC Itingen
Conditions	Standard Laboratory Conditions. Air-conditioned with target ranges for room temperature 22 ± 3 °C, relative humidity 30 - 70 % and 10 - 15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. There was a 12 hour fluorescent light / 12 hour dark cycle with at least 8 hours music during the light period.
Accommodation	Individual in Makrolon type-2 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttens).
Diet	Pelleted standard Kliba 3433, batch no. 4/04 mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst) available <i>ad libitum</i> . Results of analyses for contaminants are archived at RCC.
Water	Community tap water from Itingen, available <i>ad libitum</i> . Results of representative bacteriological, chemical and contaminant analyses are archived at RCC.

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Sponsor's Reference Number KSL040164

REPORT

5.4 CHEMICALS

³H-methyl Thymidine

Amersham TRA 310, aqueous solution, sterilized
74 GBq/mmol (2 Ci/mmol), 37 MBq/ml (1 mCi/ml)
quantities: 9.25 MBq (250 µCi), 37 MBq (1 mCi)

Supplier

Amersham Biosciences UK Limited, Buckinghamshire
England HP7 9NA, UK

Batch number

314

Storage conditions

In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

Trichloroacetic acid

Fluka no. 91230 (min. 99.5 %)

Supplier

Fluka Chemie AG (Industriestrasse 25, CH-9471
Buchs, Switzerland)

Batch number

422767/1 41801

Expiry date

14-DEC-2006

Storage conditions

In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

Phosphate buffered saline

(1 tablet solved in 200 ml bi-distilled water)

Supplier

Fluka Chemie AG (Industriestrasse 25, CH-9471
Buchs, Switzerland)

Batch number

434387/1 41202

Expiry date

MAR-2006

Storage conditions

In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

5.5 VEHICLES

1) N,N-Dimethylformamide (DMF)

Supplier

Merck KGaA (Frankfurter Str. 250, D-64293
Darmstadt, Germany)

Batch number

1.02937.0500

Expiry date

31-MAY-2006

Storage conditions

In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight

2) Acetone:olive oil, 4:1 (v/v)

Acetone

Supplier

Baker, P. H. Stehelin & Cie AG (Spalentorweg 62, CH-
4003 Basel, Switzerland)

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

Batch number 0310810002
Expiry date AUG-2004
Storage conditions In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

Olive oil

Supplier Roth AG (Chr. Merian-Ring 7, CH-4153 Reinach BL,
Switzerland)
Batch number 22357895
Expiry date 09-SEP-2004
Storage conditions In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

5.6 TEST ITEM

Identity [REDACTED]
Description [REDACTED]
[REDACTED] Sample number S2539801
Stability of test item Stable under storage conditions
Expiry date 01-JAN-2005
Storage conditions In the original container at room temperature
(20 °C ± 3 °C). Keep in dark.
Safety precautions Routine hygienic procedures (gloves, goggles, face
mask).

The supporting data for purity (characterisation), stability and homogeneity of the test item were not made available at the time of issuing this report and hence this information was excluded from the statement of compliance. However the sponsor is addressing this in a GLP compliant study [REDACTED] Study Reference No. AC030449.

The above test item data were provided by the Sponsor.

5.6.1 POSITIVE CONTROL ITEM

Identity ALPHA-HEXYLCINNAMALDEHYDE
Description liquid
Batch number 13102MO
Purity tech., 85 %
Stability of test item Stable under storage conditions
Expiry date 08-DEC-2005

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

Storage conditions: In the original container at room temperature
(20 °C ± 3 °C), away from direct sunlight.

Safety precautions: Routine hygienic procedures (gloves, goggles, face
mask).

These information was supplied by the supplier.

5.7 TEST ITEM FORMULATIONS PREPARATION

The test item and the positive control item ALPHA-HEXYLCINNAMALDEHYDE were placed into a volumetric flask on a tared Mettler balance, and vehicles N,N-dimethylformamide (DMF) or acetone:olive oil, 4:1 (v/v), respectively, was quantitatively added separately. The weight/volume dilutions were prepared individually.

Test item and positive control item formulations were made freshly before each dosing occasion and no more than 4 hours prior to application to the ears.

Homogeneity of the test item and positive control item in vehicles was maintained during treatment by use of a magnetic stirrer.

The test item in the study were assayed at three consecutive concentrations selected by the Sponsor, based on knowledge of the materials' toxicity.

Concentrations were in terms of material as supplied unless otherwise stated by the Sponsor.

5.8 RATIONALE

The study procedure was used to detect a possible contact allergenic potential of the test item applied.

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

6 STUDY CONDUCT

6.1 TREATMENT PROCEDURES

6.1.1 TOPICAL APPLICATION

Each test group of mice was treated by (epidermal) topical application to the dorsal surface of each ear lobe (left and right) with the test item at 0.25 %, 0.5 % and 1 % (w/v) in N,N-dimethylformamide (DMF). A further three groups of mice were treated with an equal volume of either, the positive control item dilution, the positive control vehicle (AOO) or the negative control material (DMF). The application volume, 25 μ l, was spread over the entire dorsal surface ($\varnothing \sim 8$ mm) of each ear lobe once daily for three consecutive days. A hair dryer was passed briefly over the ear's surface to prevent the loss of any of the test item applied.

6.1.2 ADMINISTRATION OF ^3H -METHYL THYMIDINE*

^3H -methyl thymidine ($^3\text{HTdR}$) was purchased from Amersham International (Amersham product code no. TRA 310; specific activity, 2 Ci/mmol; concentration, 1 mCi/ml).

Five days after the first topical application, all mice were administered with 250 μ l of 78.6 $\mu\text{Ci/ml}$ $^3\text{HTdR}$ (equal to 19.7 μCi $^3\text{HTdR}$) by intravenous injection via a tail vein.

6.1.3 DETERMINATION OF INCORPORATED $^3\text{HTdR}$ *

Approximately five hours after treatment with $^3\text{HTdR}$ all mice were euthanized by intraperitoneal injection of VETANARCOL (Veterinaria AG, Zürich).

The draining lymph nodes were rapidly excised and pooled for each individual animal (2 nodes per mouse). Single cell suspensions (phosphate buffered saline) of pooled lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze (200 μm mesh size). After washing two times with phosphate buffered saline (approx. 10 ml) the lymph node cells were resuspended in 5 % trichloroacetic acid (approx. 3 ml) and incubated at approximately +4 $^{\circ}\text{C}$ for at least 18 hours for precipitation of macromolecules. The precipitates were then resuspended in 5 % trichloroacetic acid (1 ml) and transferred to glass scintillation vials with 10 ml of 'Ultima Gold' scintillation liquid and thoroughly mixed.

The level of $^3\text{HTdR}$ incorporation was then measured on a β -scintillation counter. Similarly, background $^3\text{HTdR}$ levels were also measured in two 1ml-aliquots of 5 % trichloroacetic acid. The β -scintillation counter expresses $^3\text{HTdR}$ incorporation as the number of radioactive disintegrations per minute (DPM).

* Preparation of $^3\text{HTdR}$ solutions and $^3\text{HTdR}$ measurements at RCC Ltd, Environmental Chemistry & Pharamanalytics
No phase report of the results of the $^3\text{HTdR}$ level analysis was provided by the Principal Investigator.

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Sponsor's Reference Number KSL040164

REPORT

6.1.4 INTERPRETATION OF RAW DATA

The proliferative responses of lymph node cells is expressed as the number of radioactive disintegrations per minute per animal (DPM/mouse). The mean DPM/mouse value was calculated for each of the test and control groups. The ratio of ³HTdR incorporated into lymph node cells of test lymph nodes relative to that recorded for the relevant vehicle control lymph nodes (STIMULATION INDEX) was calculated by dividing the mean DPM/mouse for each test group by the mean DPM/mouse of the relevant vehicle control group. Before DPM/mouse values are determined, mean scintillation-background DPM will be subtracted from test and control raw data.

A test item is regarded as a sensitizer in the LLNA if the following criteria are fulfilled:

- First, that exposure to at least one concentration of the test item resulted in an incorporation of ³HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the STIMULATION INDEX (S.I.).
- Second, that the data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

6.2 OBSERVATIONS

In addition to the sensitizing reactions the following observations and data were recorded during the test and observation period:

Mortality / Viability	Twice daily from acclimatization start to the termination of in-life phase.
Body weights	On the test day 1 (prior to the 1 st application) and on the test day 6.
Clinical signs (local / systemic)	Daily from acclimatization start to the termination of in-life phase. Especially the treatment sites were recorded carefully.

6.3 STATISTICAL ANALYSIS

The mean body weights and mean DPM/mouse values for each test and control group were calculated. Standard deviations of the data used to determine these mean values were calculated.

The t-test was conducted for the assessment of significant differences between the positive control item group and its vehicle control group. The Dunnett-test was used for the assessment of the significant differences between the test item groups and the vehicle control group.

6.4 DATA COMPILATION

Body weights will be recorded on-line in RCC-TOX LIMS.

Clinical signs were compiled directly into the RCC computer system.

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Sponsor's Reference Number KSL040164

REPORT

7 RESULTS

7.1 CALCULATION AND RESULTS OF INDIVIDUAL DATA

The proliferative capacity of pooled lymph node cells was determined by the incorporation of ³H-methyl thymidine measured on a β -scintillation counter. The values measured are given in Appendix A.

Group	%(w/v)	DPM/mouse M \pm SD	S.I. (SD)	Statistical Analysis a) t-test (G = 2, N = 10, t = 2.31) b) Dunnett-test (G = 4, N = 20, t = 2.59)	
				t value	Conclusion
NCG 1	-	598 \pm 135	-	-	--
NCG 2	-	691 \pm 190	-	-	--
PCG 3	25 (HCA)	7565 \pm 1770	11.0 (2.6)	8.63 ^{a)}	**
TG 4	0.25	2059 \pm 280	3.4 (0.5)	4.03 ^{b)}	**
TG 5	0.5	2324 \pm 803	3.9 (1.3)	4.76 ^{b)}	**
TG 6	1	2691 \pm 758	4.5 (1.3)	5.77 ^{b)}	**
** significant difference at $p \leq 0.05$ (two sides)					
-- no significant difference at $p \leq 0.05$ (two sides)					
NCG 1 Vehicle group = N,N-dimethylformamide (DMF)					
NCG 2 Vehicle group = acetone:olive oil, 4:1 (v/v)					
PCG 3 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE in acetone:olive oil, 4:1 (v/v)					
TG 4-6 Test item in N,N-dimethylformamide (DMF)					
A dose-response relation was observed.					
An EC3 value could not be determined because this calculation requires an S.I. value of less than 3.					

The radioactive disintegration values for the individual treatment animals are included in Appendix A.

7.2 VIABILITY / MORTALITY

No deaths occurred during the study period.

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Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

7.3 CLINICAL SIGNS

No clinical signs were observed in any animals of the two vehicle control groups (Groups 1-2) or the three test item groups (Groups 4-6). On the second application day, a slight ear erythema was observed at both dosing sites in all mice of the positive control Group 3 (25 % HCA), persisting for a total of three days. In addition, on the third application day, a slight ear swelling was observed at both dosing sites in all mice of this group, persisting for a total of two days.

The individual clinical signs are included in Appendix B.

7.4 BODY WEIGHTS

The body weight of the animals, recorded on the test day 1 (prior to the 1st application) and on the test day 6, was within the range commonly recorded for animals of this strain and age.

The individual as well as groupwise summarised body weight values are included in Appendix C.

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164
[REDACTED]

REPORT

APPENDIX A

CALCULATION AND RESULTS OF INDIVIDUAL DATA

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

CALCULATION AND RESULTS OF INDIVIDUAL DATA

The following results were obtained:

Vehicles: 1) N,N-dimethylformamide (DMF); 2) acetone:olive oil, 4:1 (v/v)

No.	Test Item	% w/v	Group	dpm	dpm - BG #	Ln N	dpm/Mouse	dpm/Ln M (SD)	Statistical Analyses	Statistical Significance	S.I.	S.I. M	S.I. SD
--	--	--	BG I	6	--	--	--	--	--	--	--	--	--
--	--	--	BG II	9	--	--	--	--	--	--	--	--	--
1	--	--	CG1	447	439	2	439	598	--	--	--	--	--
2	--	--	CG1	561	553	2	553	135	--	--	--	--	--
3	--	--	CG1	748	740	2	740	--	--	--	--	--	--
4	--	--	CG1	745	737	2	737	--	--	--	--	--	--
5	--	--	CG1	529	521	2	521	--	--	--	--	--	--
6	--	--	CG2	545	537	2	537	691	--	--	--	--	--
7	--	--	CG2	985	977	2	977	190	--	--	--	--	--
8	--	--	CG2	522	514	2	514	--	--	--	--	--	--
9	--	--	CG2	663	655	2	655	--	--	--	--	--	--
10	--	--	CG2	779	771	2	771	--	--	--	--	--	--
11	HCA	25	PCG3	4950	4942	2	4942	7565	t-test	--	7.2	--	--
12	HCA	25	PCG3	7379	7371	2	7371	1770	(G = 2, N = 10, t = 2.31)	--	10.7	--	--
13	HCA	25	PCG3	9808	9800	2	9800	--	t = 8.63	--	14.2	11.0	2.6
14	HCA	25	PCG3	8341	8333	2	8333	--	--	--	12.1	--	--
15	HCA	25	PCG3	7385	7377	2	7377	--	--	--	10.7	--	--
16	TI	0.25	TG4	2093	2085	2	2085	2059	Dunnett -test	--	3.5	--	--
17	TI	0.25	TG4	2455	2447	2	2447	280	(G = 4, N = 20, t = 2.59)	--	4.1	--	--
18	TI	0.25	TG4	1770	1762	2	1762	--	t = 4.03	--	2.9	3.4	0.5
19	TI	0.25	TG4	2190	2182	2	2182	--	--	--	3.6	--	--
20	TI	0.25	TG4	1825	1817	2	1817	--	--	--	3.0	--	--
21	TI	0.5	TG5	1842	1834	2	1834	2324	Dunnett -test	--	3.1	--	--
22	TI	0.5	TG5	1718	1710	2	1710	803	(G = 4, N = 20, t = 2.59)	--	2.9	--	--
23	TI	0.5	TG5	3642	3634	2	3634	--	t = 4.76	--	8.1	3.9	1.3
24	TI	0.5	TG5	2562	2554	2	2554	--	--	--	4.3	--	--
25	TI	0.5	TG5	1895	1887	2	1887	--	--	--	3.2	--	--
26	TI	1	TG6	1832	1824	2	1824	2691	Dunnett -test	--	3.1	--	--
27	TI	1	TG6	2394	2376	2	2376	758	(G = 4, N = 20, t = 2.59)	--	4.0	--	--
28	TI	1	TG6	3451	3443	2	3443	--	t = 5.77	--	5.8	4.5	1.3
29	TI	1	TG6	2286	2278	2	2278	--	--	--	3.8	--	--
30	TI	1	TG6	3542	3534	2	3534	--	--	--	5.9	--	--

- no significant difference at p (0.05 (two sides)

* significant difference at p (0.05 (two sides)

BG = Background (1 ml 5 % trichloroacetic acid) in duplicate

CG = Control Group

TG = Test Group

S.I. = Stimulation Index

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REPORT

-
- a) = The mean value was taken from the figures BG I and BG II
CG1 = Vehicle group = N,N-dimethylformamide (DMF)
CG2 = Vehicle group = acetone:olive oil, 4:1 (v/v)
PCG3 = 25 % (w/v) ALPHA-HEXYLCINNAMALDEHYDE (HCA) in acetone:olive oil, 4:1 (v/v)
TG 4-6 = Test item in N,N-dimethylformamide (DMF)

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REPORT

APPENDIX B

INDIVIDUAL / SUMMARY CLINICAL SIGNS

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-IND - 1
25-MAY-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 1 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 1

NO CLINICAL SIGNS NOTED

ANIMAL 2

NO CLINICAL SIGNS NOTED

ANIMAL 3

NO CLINICAL SIGNS NOTED

ANIMAL 4

NO CLINICAL SIGNS NOTED

ANIMAL 5

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128

Report

SYM-IND - 2
25-MAY-04

CLINICAL SIGNS, DAILY
FEMALES
GROUP 2 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 6		

NO CLINICAL SIGNS NOTED		
ANIMAL 7		

NO CLINICAL SIGNS NOTED		
ANIMAL 8		

NO CLINICAL SIGNS NOTED		
ANIMAL 9		

NO CLINICAL SIGNS NOTED		
ANIMAL 10		

NO CLINICAL SIGNS NOTED		

RCC STUDY NUMBER 854128

Report

SYM-IND - 3
25-MAY-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 3 (POS. CONTROL GROUP)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
ANIMAL 11		

SKIN / FUR		
SWELLING (3)	G:11..
(EAR LEFT)		
SWELLING (3)	G:11..
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR RIGHT)		
ANIMAL 12		

SKIN / FUR		
SWELLING (3)	G:11..
(EAR LEFT)		
SWELLING (3)	G:11..
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR RIGHT)		
ANIMAL 13		

SKIN / FUR		
SWELLING (3)	G:11..
(EAR LEFT)		
SWELLING (3)	G:11..
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR RIGHT)		
ANIMAL 14		

SKIN / FUR		
SWELLING (3)	G:11..
(EAR LEFT)		
SWELLING (3)	G:11..
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR RIGHT)		
ANIMAL 15		

SKIN / FUR		
SWELLING (3)	G:11..
(EAR LEFT)		
SWELLING (3)	G:11..
(EAR RIGHT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR LEFT)		
GENERAL ERYTHEMA (4)	G:11..
(EAR RIGHT)		

G: Highest daily grades

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[REDACTED]

Report

SYM-IND - 4
25-MAY-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 4 (TEST GROUP 0.25%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 16

NO CLINICAL SIGNS NOTED

ANIMAL 17

NO CLINICAL SIGNS NOTED

ANIMAL 18

NO CLINICAL SIGNS NOTED

ANIMAL 19

NO CLINICAL SIGNS NOTED

ANIMAL 20

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-IND - 5
25-MAY-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 5 (TEST GROUP 0.5%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 21

NO CLINICAL SIGNS NOTED

ANIMAL 22

NO CLINICAL SIGNS NOTED

ANIMAL 23

NO CLINICAL SIGNS NOTED

ANIMAL 24

NO CLINICAL SIGNS NOTED

ANIMAL 25

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-IND - 6
25-MAY-04CLINICAL SIGNS, DAILY
FEMALES
GROUP 6 (TEST GROUP 1%)

SIGN (MAX.GRADE) (LOCATION)	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
--------------------------------	----------------------------------	---------------------

ANIMAL 26

NO CLINICAL SIGNS NOTED

ANIMAL 27

NO CLINICAL SIGNS NOTED

ANIMAL 28


NO CLINICAL SIGNS NOTED

ANIMAL 29

NO CLINICAL SIGNS NOTED

ANIMAL 30

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128


Report

SYM-SUM - 1
25-MAY-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 1 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE)	ACCLIMATISATION	TREATMENT
LOCATION	WEEKS: 1.....	1.....

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-SUM - 2
25-MAY-04

CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 2 (NEG. CONTROL GROUP)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
------------------------------	----------------------------------	---------------------

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-SUM - 3
25-MAY-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 3 (POS. CONTROL GROUP)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
SKIN / FUR -----		
SWELLING (3) (EAR LEFT)	G: %:11.. ..AA..
SWELLING (3) (EAR RIGHT)	G: %:11.. ..AA..
GENERAL ERYTHEMA (4) (EAR LEFT)	G: %:111.. ..AAA..
GENERAL ERYTHEMA (4) (EAR RIGHT)	G: %:111.. ..AAA..

G: Median value of the highest individual daily grades

%: Percent of affected animals (0 = less than 5%, 1 = between 5% and 15%, ..., A = more than 95%)

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-SUM - 4
25-MAY-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 4 (TEST GROUP 0.25%)

SIGN (MAX.GRADE)	ACCLIMATISATION	TREATMENT
LOCATION	WEEKS: 1.....	1.....

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-SUM - 5
25-MAY-04

CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 5 (TEST GROUP 0.5%)

SIGN (MAX.GRADE) LOCATION	ACCLIMATISATION WEEKS: 1.....	TREATMENT 1.....
------------------------------	----------------------------------	---------------------

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
[REDACTED]

Report

SYM-SUM - 6
25-MAY-04CLINICAL SIGNS, DAILY (SUMMARY)
FEMALES
GROUP 6 (TEST GROUP 1%)SIGN (MAX.GRADE)
LOCATIONACCLIMATISATION
WEEKS: 1.....TREATMENT
1.....

NO CLINICAL SIGNS NOTED

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

APPENDIX C

INDIVIDUAL / SUMMARY BODY WEIGHTS

RCC STUDY NUMBER 854128
[REDACTED]

Report

BW-IND - 1
25-MAY-04BODY WEIGHTS (GRAM)
FEMALES

	TREATMENT	
	1	6
DAYS	1	6
WEEKS	1	1
ANIMAL		

GROUP 1 (NEG. CONTROL GROUP)

1	21.5	22.8
2	20.7	21.7
3	20.7	22.9
4	21.3	22.2
5	20.6	21.3

GROUP 2 (NEG. CONTROL GROUP)

6	20.4	20.9
7	20.8	21.7
8	18.6	19.6
9	21.5	22.2
10	20.1	19.6

GROUP 3 (POS. CONTROL GROUP)

11	21.5	22.3
12	17.9	19.1
13	20.8	22.0
14	19.8	21.0
15	20.8	22.9

GROUP 4 (TEST GROUP 0.25%)

16	22.0	22.8
17	20.7	21.4
18	21.2	22.8
19	21.0	22.6
20	20.4	22.1

GROUP 5 (TEST GROUP 0.5%)

21	17.4	19.8
22	20.8	21.7
23	21.0	21.5
24	19.2	21.1
25	19.1	19.8

GROUP 6 (TEST GROUP 1%)

26	20.1	21.0
27	19.3	20.0
28	19.0	19.8
29	19.7	20.8
30	20.2	21.5

RCC STUDY NUMBER 854128

Report


BW-SUM - 1
25-MAY-04BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 NEG. CONTROL GROUP	GROUP 2 NEG. CONTROL GROUP	GROUP 3 POS. CONTROL GROUP
DAY 1	MEAN	20.9	20.3	20.2
WEEK 1	ST.DEV.	0.4	1.1	1.4
	N	5	5	5

		GROUP 4 TEST GROUP 0.25%	GROUP 5 TEST GROUP 0.5%	GROUP 6 TEST GROUP 1%
	MEAN	21.1	19.5	19.7
	ST.DEV.	0.6	1.4	0.5
	N	5	5	5

		GROUP 1 NEG. CONTROL GROUP	GROUP 2 NEG. CONTROL GROUP	GROUP 3 POS. CONTROL GROUP
DAY 6	MEAN	22.2	20.8	21.4
WEEK 1	ST.DEV.	0.7	1.2	1.5
	N	5	5	5

		GROUP 4 TEST GROUP 0.25%	GROUP 5 TEST GROUP 0.5%	GROUP 6 TEST GROUP 1%
	MEAN	22.3	20.8	20.6
	ST.DEV.	0.6	0.9	0.7
	N	5	5	5

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164


REPORT

APPENDIX D

GOOD LABORATORY PRACTICE

- STATEMENT OF COMPLIANCE (PRINCIPAL INVESTIGATOR)
- QUALITY ASSURANCE UNIT (PRINCIPAL INVESTIGATOR)

RCC STUDY NUMBER 854128
[REDACTED]

REPORT

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

RCC Study Number: 854128

Study Director: Dr. W. Wang-Fan, Toxicology

Test Item: [REDACTED]

Principal Investigator
³HTdR Determination: Dr. R. Burri, Environmental Chemistry & Pharmacanalytics

Phase to: [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The preparation of the [methyl-³H]Thymidine solution and determination of radioactivity content were conducted in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Principal Investigator
³HTdR Determination: Dr. R. Burri

R. Burri
.....
Date:

May 26, 2004

RCC STUDY NUMBER 854128

REPORT

QUALITY ASSURANCE

RCC Ltd, Environmental Chemistry & Pharamanalytics, CH-4452 Itingen / Switzerland

STATEMENT

RCC Study Number: 854128

Study Director: Dr. W. Wang-Fan, Toxicology

Test Item: [REDACTED]

Principal Investigator
³HTdR Determination: Dr. R. Burri, Environmental Chemistry & Pharamanalytics

Phase to: [REDACTED]
Local Lymph Node Assay (LLNA) in Mice
(Identification of Contact Allergens)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected by the quality assurance. The date is given below.

Dates and Types of QA Inspections		Dates of Reports to the Principal Investigator and to the Management
May 21, 2004	Process based (Preparation of application solution)	May 21, 2004

Sections of the draft study plan relating to the phase were reviewed and reported to the study director, lead QA and test facility management on April 29 2004

Summary report(s) of study related inspection(s) (if applicable) were issued to the study director, lead QA and test facility management.

Quality Assurance:

Mr. Jürgen Lütte

Date:

Jürgen Lütte
May 26, 2004

RCC STUDY NUMBER 854128
Sponsor's Reference Number KSL040164

REPORT

APPENDIX E

GLP - CERTIFICATION

RCC STUDY NUMBER 854128
 Sponsor's Reference Number KSL040164

REPORT

The Swiss GLP Monitoring Authorities



Swiss Federal
Office of
Public Health



Swiss Agency for the
Environment, Forests
and Landscape



Intercantonal Office
for the Control of
Medicines

Statement of GLP Compliance

It is hereby confirmed that

during the period of

August 15 – 17, 2000
 August 28 - 29, 2001 and
 April 15, 2002

the following Test Facilities of

RCC Ltd
 4452 Itingen
 Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for the Environment, Forests and Landscape and the Intercantonal Office for the Control of Medicines with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities

areas of expertise*

- Toxicology Division

TOX, ACC, MUT

- Environmental Chemistry and
Pharmanalytics Division

ACC, ECT, ENF, EMN,
PCT, RES, OTH (Animal
metabolism)

- Microbiological Diagnostics by
Biotechnology & Animal Breeding Division

OTH (Microbiology)

The inspection was performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, May 2002

Prof. Th. Zeltner

* TOX = Toxicology; ACC = Analytical and Clinical Chemistry; ECT = Environmental toxicity on aquatic and terrestrial organisms; ENF = Behaviour in water, soil and air, Bioaccumulation; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing; RES = Residue studies; OTH = Other, to be specified.

RCC Study Number 854240

[REDACTED] Study Reference Number KSG040165

[REDACTED]

Contact Hypersensitivity in Albino Guinea
Pigs, Bühler Test

Report

Author: M. Ott

Sponsor: [REDACTED]

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 Study Reference Number KSG040165

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Study Reference Number KSG040165

1 PREFACE

1.1 GENERAL

Title

Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

Sponsor

Project Planing
Contact NamesMrs E. Selbie
Mrs C. Talbot
Miss S. Buljeeon

Scientific Representative

Ms. K. Wilson

Test Facility

RCC Ltd
Toxicology
Wölferstrasse 4
CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	M. Ott
Deputy for Study Director	G. Arcelin
Technical Coordinator	P. Reissbrodt
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date	02-JUN-2004
Experimental Completion Date	09-JUL-2004
Delivery of the Animals	02-JUN-2004
Acclimatization (main study)	02-JUN-2004 to 07-JUN-2004
Observation	02-JUN-2004 to 09-JUL-2004
Treatment (main study)	08-JUN-2004 to 06-JUL-2004

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SEAC Study Reference Number KSG040165

Termination 09-JUL-2004

Study Completion Date 10-AUG-2004

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data, sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent. The remaining test item will be returned to the Sponsor. Archiving of the test items is the responsibility of the Sponsor.

RCC STUDY NUMBER 854240
SEAC Study Reference Number KSG040165

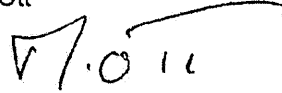
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1.5 SIGNATURE PAGE

Study Director:

M. Ott



date: 10-AUG-2004

Management:

(for) Dr. H. Fankhauser



date: 10-AUG-2004

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Study Reference Number KSG040165

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1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Ittingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 854240

TEST ITEM :

STUDY DIRECTOR : M. Ott

TITLE :

Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures with exception of the formulation trials were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
01-JUN-2004 Study Plan	01-JUN-2004
30-JUN-2004 Process Based (Test System, Test Item, Raw Data)	30-JUN-2004
10-AUG-2004 Report	10-AUG-2004

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

G. Hohl

date:

10-AUG-2004

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GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER : 854240

TEST ITEM :

STUDY DIRECTOR : M. Ott

TITLE :

Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

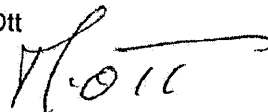
The formulation trials were performed before the study initiation date. Therefore, they are excluded from this statement.

The supporting data for purity (characterisation) was not made available at the time of issuing this report and hence this information has been excluded from the statement of compliance. However the sponsor has addressed this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director:

M. Ott



date: 10-AUG-2004

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1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

Commission Directive 96/54/EC of 30 July 1996, adapting to technical progress for the 22nd time Council Directive 67/548/EEC. Official journal No. L248, Annex IVC, B.6 "Skin Sensitization" and Annex V, section 3.2.7.2.

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 61.

1.10 CLASSIFICATION GUIDELINES

The evaluation of the results is based on the criteria of the Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. A potential contact sensitizer is classified as any article that produces in a non-adjuvant assay at least 15 % of test animals with allergic contact dermatitis. The test item will be then classified as "may cause sensitization by skin contact" and labelled with the risk phrase R43.

1.11 REFERENCES

Ritz, H.L. and Bühler, E.V.

Current Concepts Cutaneous Toxicity, ed. Drill, V.A. and Lazar, T. (Academic Press, 1980) pp. 25-40: Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests.

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Study Reference Number KSG040165

2 SUMMARY

The purpose of this skin sensitizing study was to assess the possible allergenic potential of [REDACTED] when administered topically to albino guinea pigs.

For this purpose the "Bühler Test" modified by Ritz, H.L. and Bühler, E.V. (1980) was used. Twenty female animals of the test group were treated topically with [REDACTED] at 50 % in purified water once a week for a 3-week induction phase. Two weeks after the final induction application the animals were challenged with the test item concentration of 25 % in purified water.

The ten animals of the control group were not treated during the induction. They were treated once at challenge with [REDACTED] at 25 % in purified water.

Results

None of the control or test animals were observed with skin reactions after challenge treatment with the highest tested, non-irritating concentration of [REDACTED] (25 % in purified water).

PRIMARY SENSITIZATION RESULTS (INCIDENCE TABLES)

CHALLENGE

The highest tested non-irritating concentration of [REDACTED] used for challenge was 25 % in purified water. The incidence of positive erythema reactions after topical challenge is described as follows:

ERYTHEMA SCORE	TEST GROUP 20 animals		CONTROL GROUP 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	17	17	10	10
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
No. with grades ≥ 1	0	0	0	0
No. tested	17	17	10	10
INCIDENCE*	0/17***		0/10	
SEVERITY**	0		0	

* Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

** Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

*** Three animals of the test group were found dead on test days 10 and 11 (i.e. two and three days after the second induction application). At necropsy a number of macroscopic findings were recorded in these three animals including hemorrhagic lungs, congested (not collapsed) lungs with dense parenchymal focus/foci, enlarged spleen and stomach distended with gas. The cause of death was not established but there are historical incidences of spontaneous death in the Buehler test (with the same macroscopic findings on necropsy), in both treated and control animals, which indicates that the deaths in this study were not treatment related.

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[REDACTED]
Study Reference Number KSG040165

3 CONCLUSION

In this study none of the animals of the control and test group were observed with skin reactions after challenge treatment performed with the highest tested non-irritating concentration of [REDACTED] at 25 % in purified water.

Based on the above mentioned findings in a non-adjuvant sensitization test in guinea pigs and in accordance to Commission Directive 2001/59/EC, [REDACTED] applied at a concentration of 25 % in purified water does not have to be classified and labelled as a skin sensitizer.

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Study Reference Number KSG040165

4 PURPOSE

The purpose of this skin sensitization study was to determine if [REDACTED] under the conditions described in the study plan and this report, causes an increased reaction in the skin of guinea pigs at challenge when compared to appropriate controls.

This study should provide a rational basis for risk assessment of the sensitizing potential of the test item in man.

The sensitivity and reliability of the experimental technique employed was assessed by use of ALPHA-HEXYLCINNAMALDEHYDE which is recommended by the OECD 406 Guidelines and is known to have moderate skin sensitization properties in the guinea pig strain. The results from the most recent test run (RCC study number 851772, performed from 25-NOV-2003 to 02-JAN-2004) are included in this report under the APPENDIX F.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF
Rationale	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (<i>in-vitro</i>) is available at present for the test of contact sensitization.
Source	Harlan Netherlands BV Kreuzelweg 53 NL-5961 NM Horst / The Netherlands Postbus 6174 NL-5960 AD Horst / The Netherlands
Number of animals for main study / Irritation screen	30 females / 4 females (nulliparous and non-pregnant) Challenge: - 20 test animals - 10 control animals Irritation Screen: - 4 animals
Age at delivery/ acclimatization start	4 - 6 weeks
Body weight at delivery/ acclimatization start	Test and control animals: 287 - 366 g Animals used for irritation screen: 285 - 355 g
Identification	By unique cage number and corresponding individual animal number.
Randomization	Randomly selected by hand at time of delivery. No computer randomization.

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Acclimatization

Under test conditions after health examination. Six days for the control and test group. However, contrary to the test group the control group remained untreated during the 3 induction weeks.

One day for the animals used in the irritation screen for induction and challenge. Only animals without any visible signs of illness were used for the study.

5.2 ALLOCATION

The animals were distributed as follows:

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP
1 Irritation Screen for Induction and Challenge	4	153 - 156
2 Control Group	10	157 - 166
3 Test Group	20	167 - 186

5.3 HUSBANDRY**Room no.**

103 / RCC Ltd, Füllinsdorf

Conditions**Standard Laboratory Conditions**

Air-conditioned with target ranges for room temperature 22 ± 3 °C, relative humidity 30-70 % and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the daytime light period.

Accommodation

Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz).

Diet

Pelleted standard Provimi Kliba 3418, batch nos. 24/04 and 33/04 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, CH-4303 Kaiseraugst), *ad libitum*. Results of analyses for contaminants are archived at RCC Ltd.

Water

Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd.

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5.4 TEST ITEM

The following information was provided by the sponsor:

Identification

Description

Batch number

S2539801

Purity / Formulation

The purity of the test item was not available prior to administration, hence this information is excluded from the Statement of Compliance. However, the Sponsor is addressing this in a GLP compliant study [REDACTED] Study Reference Number AC030449.

Stability of test item

Stable under storage conditions;
expiration date: 01-JAN-2005

Stability of test item dilution

Stable in purified water for at least 7 days in the refrigerator.

Storage conditions

At room temperature (range of 20 ± 3 °C), light protected.

Safety precautions

Routine hygienic procedures were used to ensure the health and safety of the personnel.

Characterization, stability and homogeneity are being addressed by the Sponsor in a GLP compliant study [REDACTED] Study Reference Number AC030449.

5.5 VEHICLE

The following information was provided by RCC Ltd:

Purified water prepared at RCC Ltd (deionised water which was processed and treated by the PURELAB Option-R unit. This latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).

The vehicle was selected based on preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials were excluded from the statement of GLP compliance. Purified water was a suitable vehicle to be used for the study.

5.6 TEST ITEM PREPARATION

The test item and vehicle were placed into a glass beaker on a tared Mettler PM 460 balance and weight/weight dilutions were prepared. Homogeneity of the test item in purified water was ensured and maintained during treatment using a magnetic stirrer. The preparations were made immediately prior to each dosing.

Dose levels were in terms of material as supplied by the sponsor.

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5.7 RATIONALE

Dermal administration has historically been used as the route of choice for determining delayed contact hypersensitivity.

5.8 SELECTION OF CONCENTRATION OF TEST ITEM FOR MAIN STUDY

A number of factors contributed to the selection of the concentrations of test item including irritancy, slope of dose response curve and experience with similar test items. Selection was based on the following criteria:

Epidermal Induction: Concentration that produced some irritation but not adversely affected the animals (determined at the irritation screen).

Epidermal Challenge: Concentration that was the maximum tested non-irritant concentration (determined at the irritation screen).

5.9 GRADING METHOD

The test item skin area of the animals used for irritation screen and challenge were depilated approximately 21 hours after the patches had been removed, using an approved depilatory cream (VEET Cream, Reckitt & Colman AG, CH-4123 Allschwil). The depilation was performed to clean the stratum corneum from the remnants produced by the test item and to facilitate the reading of the skin reactions. The depilatory cream was placed on the patch sites and surrounding areas, and left on for up to 3-5 minutes. It was then thoroughly washed off with a stream of warm, running water. The animals were then dried with a disposable towel, and returned to their cages.

The scoring system was performed by visual assessment of erythema, oedema and other clinical changes in skin conditions. They were assessed as follows:

- 0 = no visible change
- 1 = discrete or patchy erythema
- 2 = moderate and confluent erythema
- 3 = intense erythema and swelling

Grading of all animals was done by positioning each animal under true-light (Philips TLD 36W/84 or Osram 36W/31 830).

The grading method used for irritation screen, induction and challenge was identical. It was performed 24 hours (± 10 minutes) after removal of the patches for irritation screen, induction and challenge and repeated 24 ± 4 hours later (48-hour grades) for the irritation screen and the challenge.

Note: At challenge, control animals were graded before the test animals.

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5.10 TREATMENT METHODS

Patching method: The same patching method was used for irritation screen, induction and challenge.

The animal's fur was shaved with a fine clipper blade just prior to the exposure. Closed patches were applied to the animals as follows:

0.5 mL of the freshly prepared test item solution in a 25 mm Hill Top Chamber.

The 25 mm Hill Top Chamber was firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressing was left in place for six hours (\pm 10 minutes).

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6 STUDY CONDUCT - TREATMENT PROCEDURE

6.1 DIAGRAMMATIC STUDY PLAN

Acclimatization		Study day				
-6	-5	1	8	15	22	29
IS		I	I	I		C

IS = Irritation screen to determine the minimal irritating concentration used in the induction period and the highest non-irritating concentration used for the challenge.

I = Induction (test group only)

C = Challenge (control and test group)

6.2 IRRITATION SCREEN FOR INDUCTION AND CHALLENGE – PERFORMED DURING THE ACCLIMATIZATION PERIOD

The test item concentrations described below were selected during a preliminary solubility testing which was performed before the study initiation date.

For patch placements, the format described below was used on 4 guinea pigs. Four different concentrations were used on each animal for a 6-hour exposure period.

Test Item	Concentrations		Vehicle	Formulation
[REDACTED]	A = 50 %	C = 10 %	purified water	weight/weight
	B = 25 %	D = 5 %		

cranial		cranial		cranial		cranial	
left	A C	right	left	D B	right	left	C A
	B D			A C			D B
caudal		caudal		caudal		caudal	
Animal no. 153		Animal no. 154		Animal no. 155		Animal no. 156	

The allocation of the different test item dilutions to the sites (A, B, C, D) on the four animals was alternated in order to minimize site-to-site variation in responsiveness.

The application sites were assessed for erythema and oedema 24 and 48 ± 4 hours after removal of the patches.

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The results are described on page 24 and are summarized as follows:

Response Grade	Irritancy Results							
	after the 24-hour reading concentration (%) of				after the 48-hour reading concentration (%) of			
	50 %	25 %	10 %	5 %	50 %	25 %	10 %	5 %
0	0*	4	4	4	2	4	4	4
1	4	0	0	0	2	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0

* = number of grade-related skin response

A concentration of 50% in purified water caused some irritation without adversely affecting the animals and was therefore chosen as the most appropriate concentration to stimulate a state of immune hypersensitivity during the induction phase. The highest non-irritating concentration tested was 25% in purified water and this was chosen as the challenge concentration.

6.3 INDUCTION – PERFORMED ON TEST DAYS 1, 8 AND 15

The fur was clipped from the left shoulder of each test animal and the patches applied, over a period of 3 weeks. Each animal received one patch per week with the test item at 50 % in purified water which remained in place for 6 hours each. The repeated application was performed at the same site. The interval between exposure was one week. The control animals remained untreated.

After the last induction exposure the test animals were left untreated for 2 weeks before the challenge.

The skin responses were graded 24 hours (\pm 10 minutes) after the patches had been removed.

Any gross skin reactions were recorded without depilation.

6.4 CHALLENGE – PERFORMED ON TEST DAY 29

The animals previously exposed during the induction period (i.e. test group) as well as the previously untreated control animals were challenged two weeks after the last induction exposure using the test item at 25 % in purified water. The fur was clipped from the left posterior quadrant of the side and back of the animals. Patch sites for challenge are indicated below. The exposure period was 6 hours (\pm 10 minutes) on a naïve skin site.

The responses were graded at 24 and 48 hours (\pm 10 minutes) after the patches had been removed, according to the grading method described above.

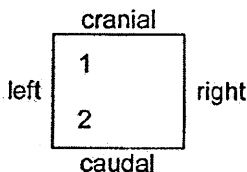
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6.5 FORMAT FOR INDUCTION AND CHALLENGE PATCH APPLICATION



1 = Induction (test group only)

2 = Challenge (control and test group)

6.6 OBSERVATIONS

The following observations and data were recorded during the study:

Viability / Mortality	Daily from delivery of the animals to the termination of test.
Clinical signs / Grading of skin response score	Daily from delivery of the animals to the termination of test. Skin responses were graded during the irritation screen, induction and challenge period.
Body weights	At acclimatization and treatment start, and at the termination of the study.

Records were maintained of all additional and standard observations.

These observations applied to the main study groups and to the irritation screen group to the extent of their use in the study.

6.7 EVALUATION OF SKIN REACTIONS

For evaluation, two parameters were used: the incidence index and the severity index, for both test and control animals. The incidence index is an expression of the number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals in the group, while the severity index is calculated from the total sum of 24- and 48-hour response readings divided by the number of animals exposed.

In this study, the incidence and severity index are of zero.

7 PATHOLOGY

7.1 NECROPSY

Necropsy was performed on three animals (no. 167, 169, 184) of the test group which were found dead on test days 10 and 11 (i.e. two and three days after the second induction application).

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No necropsies were performed on the surviving animals of the control and test group sacrificed at termination of their observation period or on the animals of the irritation screen sacrificed on test day 1.

The surviving animals were euthanized by intraperitoneal injection of Vetanarcol at a dose of at least 2.0 mL/kg body weight (equivalent to 324 mg sodium pentobarbitone/kg body weight) and discarded.

8 STATISTICAL ANALYSIS

Descriptive statistics (means and standard deviations) were calculated for body weights. No inferential statistics were used.

9 DATA COMPILATION

The following data were recorded on data sheets and transcribed for compilation and analysis: skin reactions, viability/mortality, clinical signs.

The following data were recorded on-line: body weights.

The following data were compiled into the RCC Tox Computer System during recording: macroscopic findings.

The RCC Tox Computer System (RCC-Tox-Lims) has been validated with respect to data collection, storage and retrievability.

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10 RESULTS

Main Study

10.1 VIABILITY / MORTALITY / MACROSCOPIC FINDINGS

Three animals (no. 167, 169, 184) of the test group were found dead on test days 10 and 11 (i.e. two and three days after the second induction application). At necropsy a number of macroscopic findings were recorded in these three animals including hemorrhagic lungs, congested (not collapsed) lungs with dense parenchymal focus/foci, enlarged spleen and stomach distended with gas. The cause of death was not established but there are historical incidences of spontaneous death in the Buehler test (with the same macroscopic findings on necropsy), in both treated and control animals, which indicates that the deaths in this study were not treatment related.

See p. 30

10.2 CLINICAL SIGNS, SYSTEMIC

No symptoms of systemic toxicity were observed in the animals.

10.3 SKIN EFFECT IN THE INDUCTION

Due to the yellow remnants of the test item, a possible erythema reaction could not be determined during the three weeks of induction. However, no oedema was observed. The test sites were not depilated to facilitate the reading during the three inductions unlike the irritation screen and challenge procedure. The depilation was omitted to avoid repeated mechanical irritation produced during the removal of the depilation cream and test item.

The control group remained untreated.

See p. 26

10.4 SKIN EFFECT IN THE CHALLENGE

No skin reactions were observed in the control and test animals treated with the test item at 25 % in purified water.

The control and test animals were depilated approximately 3 hours prior to the 24-hour reading to clean the test item skin area from the yellow remnants produced by the test item and to facilitate the reading of the skin reactions.

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10.5 BODY WEIGHTS

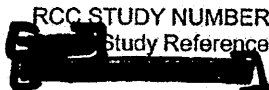
The body weight of the animals was within the range commonly recorded for animals of this strain and age.

See pp. 32 - 34

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APPENDIX A

SKIN REACTIONS DURING IRRITATION SCREEN FOR INDUCTION AND CHALLENGE

- INDIVIDUAL FINDINGS

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 Study Reference Number KSG040165

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SKIN REACTIONS DURING IRRITATION SCREEN FOR INDUCTION AND CHALLENGE – INDIVIDUAL FINDINGS

IRRITATION SCREEN

Animal No.: 153 female

	Skin reactions after	
	24 Hours	48 Hours
A = 50 %	1	1
B = 25 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
C = 10 %	0	0
D = 5 %	0	0

Animal No.: 154 female

	Skin reactions after	
	24 Hours	48 Hours
D = 5 %	0	0
A = 50 %	1	0

	Skin reactions after	
	24 Hours	48 Hours
B = 25 %	0	0
C = 10 %	0	0

Animal No.: 155 female

	Skin reactions after	
	24 Hours	48 Hours
C = 10 %	0	0
D = 5 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
A = 50 %	1	1
B = 25 %	0	0

Animal No.: 156 female

	Skin reactions after	
	24 Hours	48 Hours
B = 25 %	0	0
C = 10 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
D = 5 %	0	0
A = 50 %	1	0

Three hours prior to the 24-hour reading both flanks were depilated.

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[REDACTED] Study Reference Number KSG040165

APPENDIX B

SKIN REACTIONS OBSERVED DURING INDUCTION

- INDIVIDUAL FINDINGS

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Study Reference Number KSG040165

SKIN REACTIONS OBSERVED DURING INDUCTION – INDIVIDUAL FINDINGS

INDUCTION WEEK 1 / application on test day 1

Test item concentration: 50 %
Vehicle: Purified water

TEST GROUP

Animal number female	167	168	169	170	171	172	173	174	175	176
Skin reaction*	-	-	-	-	-	-	-	-	-	-

Animal number female	177	178	179	180	181	182	183	184	185	186
Skin reaction*	-	-	-	-	-	-	-	-	-	-

INDUCTION WEEK 2 / application on test day 8

Test item concentration: 50 %
Vehicle: Purified water

TEST GROUP

Animal number female	167	168	169	170	171	172	173	174	175	176
Skin reaction*	-	-	-	-	-	-	-	-	-	-

Animal number female	177	178	179	180	181	182	183	184	185	186
Skin reaction*	-	-	-	-	-	-	-	-	-	-

INDUCTION WEEK 3 / application on test day 15

Test item concentration: 50 %
Vehicle: Purified water

TEST GROUP

Animal number female	167	168	169	170	171	172	173	174	175	176
Skin reaction*	exitus	-	exitus	-	-	-	-	-	-	-

Animal number female	177	178	179	180	181	182	183	184	185	186
Skin reaction*	-	-	-	-	-	-	-	exitus	-	-

* Due to yellow remnants produced by the test item a possible erythema reaction could not be determined. However, no oedema was observed. The animals were not depilated.

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APPENDIX C

SKIN REACTIONS AFTER CHALLENGE

- INDIVIDUAL FINDINGS

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SKIN REACTIONS AFTER CHALLENGE – INDIVIDUAL FINDINGS

Test item:

Test item concentration:

25 %

Vehicle:

Purified water

CONTROL GROUP

Animal No. female	Skin Reactions after (± 2 Hours)	
	24 Hours	48 Hours
157	0	0
158	0	0
159	0	0
160	0	0
161	0	0

Animal No. female	Skin Reactions after (± 2 Hours)	
	24 Hours	48 Hours
162	0	0
163	0	0
164	0	0
165	0	0
166	0	0

TEST GROUP

Animal No. female	Skin Reactions after (± 2 Hours)	
	24 Hours	48 Hours
167	EXITUS	EXITUS
168	0	0
169	EXITUS	EXITUS
170	0	0
171	0	0
172	0	0
173	0	0
174	0	0
175	0	0
176	0	0

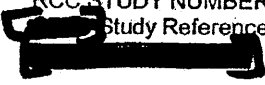
Animal No. female	Skin Reactions after (± 2 Hours)	
	24 Hours	48 Hours
177	0	0
178	0	0
179	0	0
180	0	0
181	0	0
182	0	0
183	0	0
184	EXITUS	EXITUS
185	0	0
186	0	0

Approximately 3 hours prior to the 24-hour reading, the test item-treated flank was depilated.

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APPENDIX D

NECROPSY

- MACROSCOPIC FINDINGS

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NECROPSY – MACROSCOPIC FINDINGS**MACROSCOPICAL FINDINGS****FEMALES****GROUP 3 (TEST GROUP)**

ANIMAL 167

(SPONTANEOUS DEATH, 18-JUN-04)

LUNGS..... HEMORRHAGIC.

SPLEEN..... ENLARGED.

ANIMAL 169

(SPONTANEOUS DEATH, 18-JUN-04)

LUNGS..... HEMORRHAGIC.

STOMACH..... DISTENDED WITH GAS.

ANIMAL 184

(SPONTANEOUS DEATH, 17-JUN-04)


LUNGS..... NOT COLLAPSED.
CONGESTION.
DENSE PARENCHYMAL FOCUS/FOCI.

SPLEEN..... ENLARGED.

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APPENDIX E

BODY WEIGHTS

- SUMMARY

- INDIVIDUAL

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BODY WEIGHTS – SUMMARY**BODY WEIGHTS (GRAM) SUMMARY
FEMALES**

ACCLIMATIZATION		GROUP 1	GROUP 2	GROUP 3
		IRRITATION SCREEN	CONTROL GROUP	TEST GROUP
DAY 1	MEAN	305	347	319
WEEK 1	ST. DEV.	33.3	13.3	27.0
	MINIMUM	285	325	287
	MAXIMUM	355	366	355
	N	4	10	20

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BODY WEIGHTS – SUMMARY (CONTINUED)

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 IRRITATION SCREEN	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY 1	MEAN	355	428	397
WEEK 1	ST. DEV.	41.4	16.9	32.8
	MINIMUM	315	392	345
	MAXIMUM	413	448	447
	N	4	10	20
DAY 32	MEAN	---	640	619
WEEK 5	ST. DEV.	---	49.3	38.4
	MINIMUM	---	532	548
	MAXIMUM	---	699	683
	N	0	10	17

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BODY WEIGHTS – INDIVIDUAL

BODY WEIGHTS (GRAM)
FEMALES

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	32
WEEKS	1	1	5
ANIMAL			

GROUP 1 (IRRITATION SCREEN)

153	293	339	---
154	288	315	---
155	285	352	---
156	355	413	---

GROUP 2 (CONTROL GROUP)

157	366	448	699
158	337	429	634
159	350	433	647
160	331	443	686
161	347	411	662
162	356	427	635
163	325	392	532
164	341	437	625
165	352	420	599
166	362	441	685

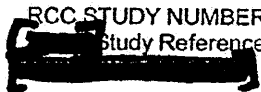
GROUP 3 (TEST GROUP)

167	354	425	---
168	350	421	662
169	351	447	---
170	335	434	628
171	355	428	625
172	294	345	616
173	290	368	632
174	312	363	617
175	300	388	647
176	352	438	683
177	343	432	620
178	354	439	669
179	323	404	574
180	292	378	580
181	292	382	596
182	296	351	548
183	298	369	560
184	287	378	---
185	304	366	606
186	294	391	656

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APPENDIX F

RESULTS OF POSITIVE CONTROL

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RESULTS OF POSITIVE CONTROL

RCC Study Number 851772

ALPHA-HEXYLCINNAMALDEHYDE:

Contact Hypersensitivity in Albino Guinea
Pigs, Bühler Test

POSITIVE CONTROL

performed from 25-NOV-2003 to 02-JAN-2004

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RESULTS OF POSITIVE CONTROL (CONTINUED)

SUMMARY

For validation of the sensitivity of test method and test system used, a known moderate sensitizer ALPHA-HEXYLCINNAMALDEHYDE was selected as a positive control. This was performed from 25-NOV-2003 to 02-JAN-2004 in accordance with the recommendation of:

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

The raw data from this study are kept in a separate file at RCC Ltd, CH-4452 Itingen. The test described was performed under GLP-conditions with a final QA-check.

TEST ITEM

Identification	ALPHA-HEXYLCINNAMALDEHYDE (HCA)
Description	Yellow liquid
Supplier	Aldrich Chemical Company Inc. P.O. Box 260 CH-9471 Buchs SG / Switzerland
Date of test item receipt	07-SEP-2001
Lot number	01016AQ
Purity	87.8 % (certificate of analysis to be retained as data)
Stability of test item	Stable under storage conditions; expiration date: 07-SEP-2004
Stability of test item dilution	Unknown in PEG 300.
Storage conditions	At room temperature (range of $20 \pm 3^{\circ}\text{C}$), light protected.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

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RESULTS OF POSITIVE CONTROL (CONTINUED)**VEHICLE**

Identification	Polyethylene glycol 300 (PEG 300)
Description	Colorless viscous liquid
Lot number	448174/1 21203148
Source	FLUKA Chemie GmbH, CH-9471 Buchs
Stability of vehicle	Stable under storage conditions; expiration date: 16-APR-2005
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), light protected.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

TEST SYSTEM

Test system	Ibm: GOH1; SPF-quality guinea pigs (synonym: Himalayan spotted)
Rationale	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (<i>in-vitro</i>) is available at present for the test of contact sensitization.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals for main study / Irritation screen	30 females / 4 females (nulliparous and non-pregnant) Challenge: - 20 test animals - 10 control animals Irritation Screen: - 4 animals
Age at delivery / acclimatization start	4 - 6 weeks
Body weight at delivery / acclimatization start	Test and control animals: 348 - 414 g Animals used for irritation screen: 366 - 381 g
Identification	By unique cage number and corresponding individual animal number.
Randomization	Randomly selected by hand at time of delivery. No computer randomization.

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RESULTS OF POSITIVE CONTROL (CONTINUED)

Acclimatization

Under test conditions after health examination. One week for the control and test group. However, contrary to the test group the control group remained untreated during the 3 induction weeks.

One day for the animals used in the irritation screen for induction and challenge. Only animals without any visible signs of illness were used for the study.

The purpose of this skin sensitizing study was to confirm the possible allergenic potential of ALPHA-HEXYLCINNAMALDEHYDE and to prove the sensitivity of the test system when administered topically to albino guinea pigs.

For this purpose the "Bühler Test" modified by Ritz, H.L. and Bühler, E.V. (1980) was used. Twenty female animals of the test group were treated topically with ALPHA-HEXYLCINNAMALDEHYDE at 50 % in PEG 300 once a week for a 3-week induction phase. Two weeks after the final induction application the animals were challenged with the test item concentration of 5 % in PEG 300.

The ten animals of the control group were not treated during the induction. They were treated once at challenge with ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300.

Results

Twenty (at the 24-hour reading) and seventeen (at the 48-hour reading) out of 20 test animals were observed with discrete/patchy to moderate/confluent erythema after the challenge treatment with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300. No skin effect was observed in the control group.

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RESULTS OF POSITIVE CONTROL (CONTINUED)**PRIMARY SENSITIZATION RESULTS (INCIDENCE TABLES)****CHALLENGE**

The highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE used for challenge was 5 % in PEG 300. The incidence of positive erythema reactions after topical challenge is described as follows:

ERYTHEMA SCORE	TEST GROUP 20 animals		CONTROL GROUP 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	0	3	10	10
1	8	13	0	0
2	12	4	0	0
3	0	0	0	0
No. with grades ≥ 1	20	17	0	0
No. tested	20	20	10	10
INCIDENCE*	20/20		0/10	
SEVERITY**	1.1 – 1.6		0	

* Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

** Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

CONCLUSION

In this study, 100 % (at the 24-hour reading) of the animals of the test group were observed with skin reactions after challenge treatment performed with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300.

No skin reactions were observed in the control group treated in the same conditions during the challenge phase.

Based on the above mentioned findings in a non-adjuvant sensitization test in guinea pigs and in accordance to Commission Directive 2001/59/EC, ALPHA-HEXYLCINNAMALDEHYDE applied at a concentration of 5 % in PEG 300 does have to be classified and labelled as a skin sensitizer and proved the sensitivity of the test system.

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RESULTS OF POSITIVE CONTROL (CONTINUED)

CHALLENGE

Test item: ALPHA-HEXYLCINNAMALDEHYDE
 Test item concentration: 5 %
 Vehicle: PEG 300

CONTROL GROUP

Animal No. female	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
508	0	0
509	0	0
510	0	0
511	0	0
512	0	0

Animal No. female	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
513	0	0
514	0	0
515	0	0
516	0	0
517	0	0

TEST GROUP

Animal No. female	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
518	1	1
519	2	1
520	2	1
521	1	0
522	1	1
523	2	1
524	2	2
525	2	1
526	1	0
527	1	1

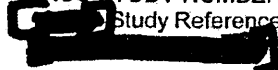
Animal No. female	Skin Reactions after (\pm 2 Hours)	
	24 Hours	48 Hours
528	2	1
529	2	1
530	2	1
531	2	2
532	2	2
533	1	0
534	2	1
535	1	1
536	1	1
537	2	2

Approximately 3 hours prior to the 24-hour reading, the test item-treated flank was depilated.

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APPENDIX G

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

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Study Reference Number KSG040165

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

Test item identification: [REDACTED]				
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (SENSITIZATION POTENTIAL BY EPICUTANEOUS ADMINISTRATION - BÜHLER TEST)				
Batch No.:		S2539801		
RCC Study No.:		854240		
Study Completion Date: 10-AUG-2004				
Species/Strain: Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF			Number of exposed animals: 30	
Procedure	Administration route/site	Day	Vehicle	
Induction phase/ 6-hour application	Occl. patch/left shoulder	1, 8, 15	Purified water	
Challenge/ 6-hour application	Occl. patch/left flank	29	Purified water	
Study Group	Control Group		Test Group	
	Concentration of test item	Number of appl. and dose	Concentration of test item	Number of appl. and dose
Induction phase/ 6-hour application	---	---	50 %	1x0.5mL/week/ 25mm Hill Top Chamber
Challenge/ 6-hour application	25 %	1x0.5mL/25mm Hill Top Chamber	25 %	1x0.5mL/25mm Hill Top Chamber
Number of animals and sex	10 females		20 females	
Number of animals showing a grade ≥ 1 at either 24 or 48 hours / out of total (incidence index)				
Challenge	0/10		0/17*	
Summary of salient findings: The test item tested under the described conditions is considered not to be a skin sensitizer.				
Study in compliance with GLP:			YES: X	NO:
QA inspected/audited:			YES: X	NO:

* Three animals of the test group were found dead on test days 10 and 11.

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APPENDIX H

GLP – CERTIFICATION

RCC STUDY NUMBER 854240

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GLP – CERTIFICATION

The Swiss GLP Monitoring Authorities

Swiss Federal
Office of
Public HealthSwiss Agency for the
Environment, Forests
and Landscape

swissmedic

Swissmedic
Swiss Agency for
Therapeutic Products**Statement of GLP Compliance**

It is hereby confirmed that

during the period of

November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities**Areas of expertise *****- Toxicology**TOX, ACC, MUT,
OTH (Safety Pharmacology)**- Environmental Chemistry and
Pharmanalytics**ACC, ECT, ENF, EMN, PCT,
RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology; ACC = Analytical and Clinical Chemistry; ECT = Environmental toxicity on aquatic and terrestrial organisms; ENF = Behaviour in water, soil and air; Bioaccumulation; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing; RES = Residue studies; OTH = Other, to be specified.

RCC Study Number 851276

Sponsor Study Number KF 030425

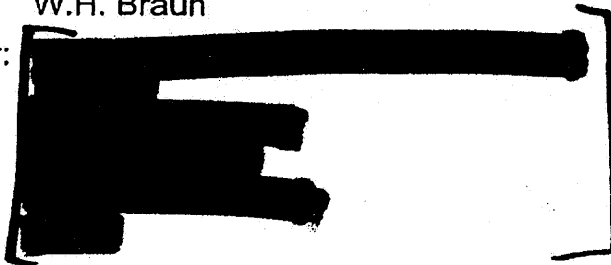


7-Day Range-Finding Oral Toxicity
(Gavage) Study in the Wistar Rat

Report

Author: W.H. Braun

Sponsor:



BCC STUDY NUMBER 851276
[REDACTED]**GENERAL**

In this dose range-finding toxicity study [REDACTED] was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 10, 100 or 1000 mg/kg body weight for a period of seven days. A control group received a similar dose volume (10 ml/kg body weight) of the vehicle, bidistilled water. The study comprised two animals per group and sex which were sacrificed after seven days of treatment. Clinical signs, food consumption and body weights were recorded periodically during the acclimatization and treatment periods. At the end of the dosing period, all animals were killed, necropsied and examined *post mortem*. The results of the study are summarized as follows:

Mortality

All control animals and all rats treated with 10 mg/kg/day and 100 mg/kg/day survived until scheduled necropsy.

One male (no. 8) and both females (nos. 15 and 16) treated with 1000 mg/kg/day were found dead on Day 1 of treatment, and the remaining male (no. 7) treated with 1000 mg/kg/day was killed for ethical reasons on same day.

Clinical Signs

One hour after administration, three rats treated with 1000 mg/kg/day were found dead. Male no. 7 had convulsive contractions and was prostrate. When observed again approximately 10 minutes later, these signs were still present and signs of somnolence were recorded. This male was sacrificed for ethical reasons.

No clinical signs were seen in rats treated with 10 mg/kg/day or 1000 mg/kg/day from days 1-7 of treatment.

Food Consumption

The mean daily food consumption of the surviving test item-treated males and females was not affected during days 1-7 of treatment.

Body Weights

The mean body weights of the remaining test item-treated males and females compared favorably to those of the respective control values.

Organ Weights

No test item-related effects on mean absolute and relative organ weights were noted after 7 days' treatment when compared with the controls.

Macroscopical Findings

Bilateral renal pelvis dilation was noted in one female (no. 12) treated with 10 mg/kg/day. This is considered to be a typical background finding and is unrelated to the treatment with the test item.

Dark red thymic foci were noted in one female (no. 16) which was found dead on treatment day 1.

All other animals were without macroscopical changes at necropsy.


RCC STUDY NUMBER 851276
[REDACTED]

ASSESSMENT

Based on the results of this seven-day dose range-finding study and discussions with the sponsor, dose levels of 1, 10 or 100 mg/kg body weight/day are proposed for the subsequent 28-day study (RCC Study Number 851277) with [REDACTED]

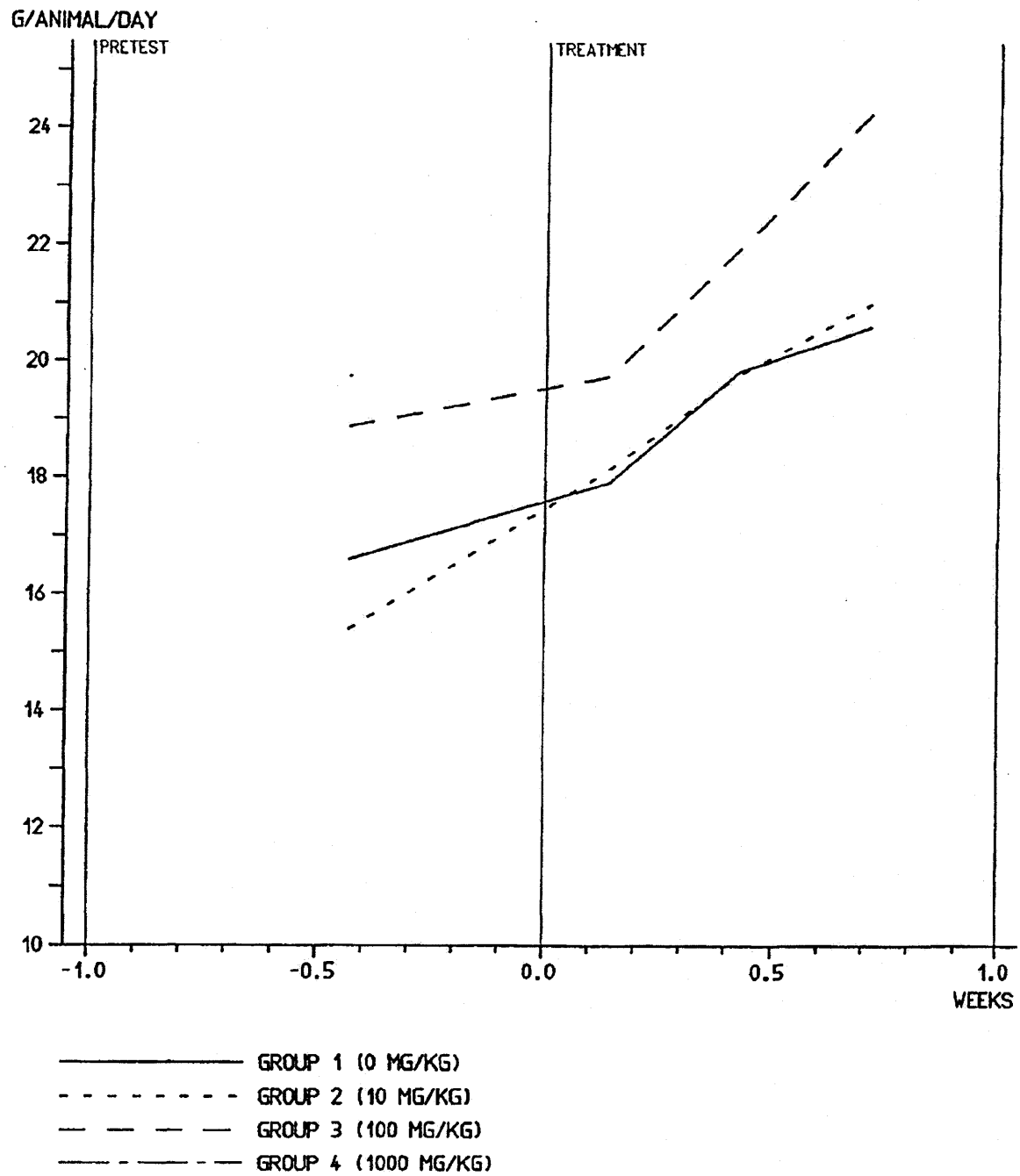
Study Director

W.H. Braun


Date: 25 Nov 2003

RCC STUDY NUMBER 851276

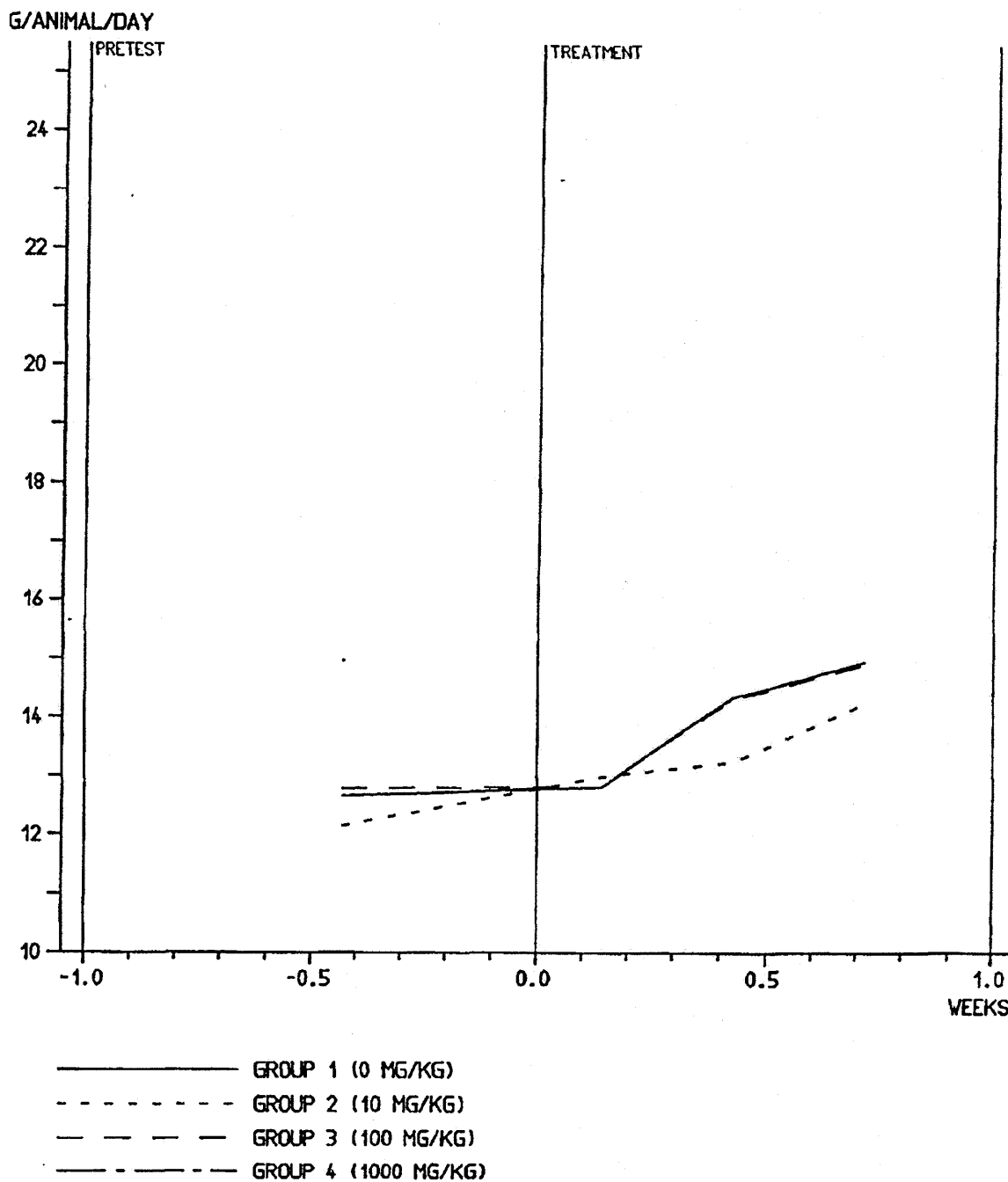
FIGURES

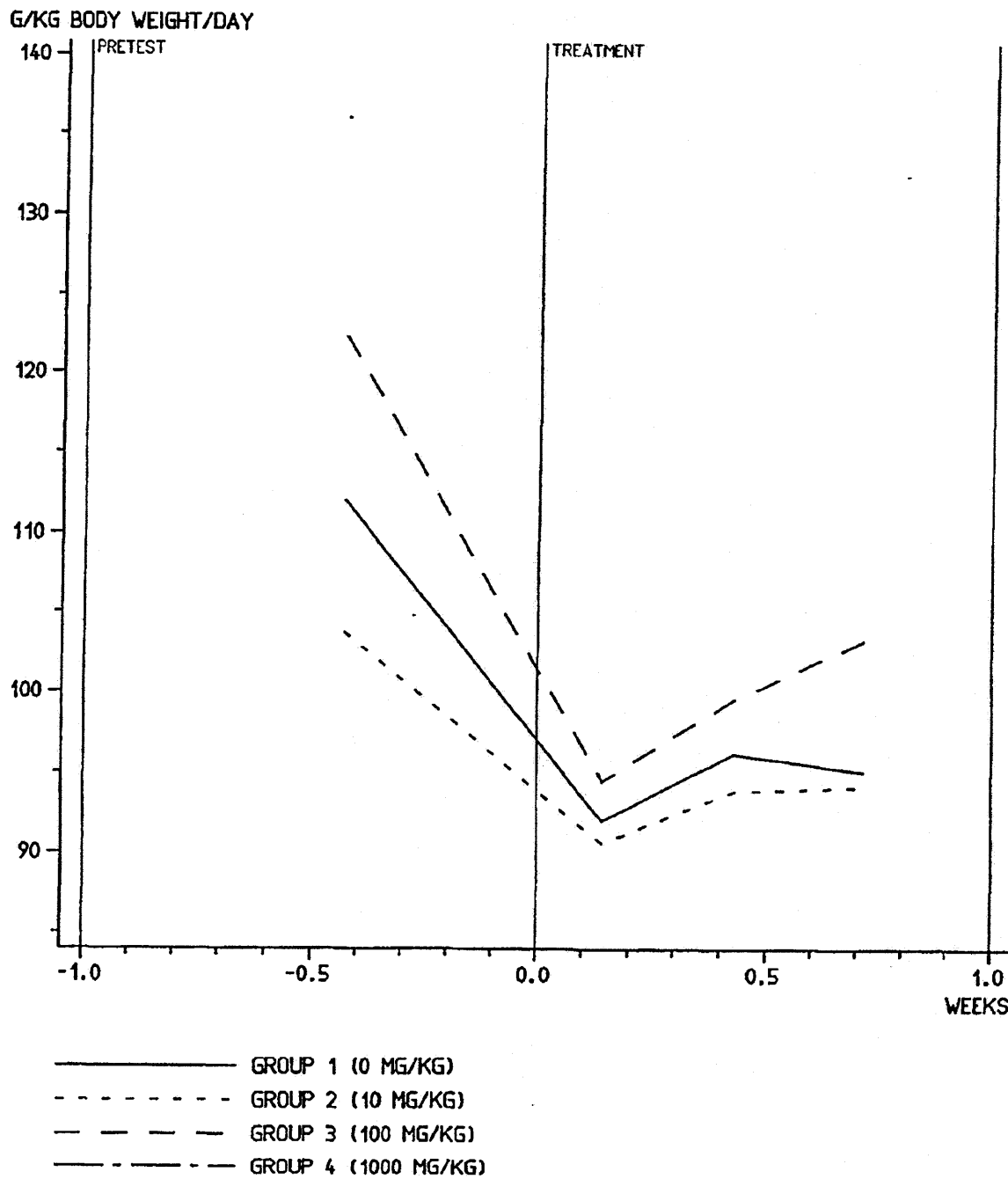
RCC STUDY NUMBER 851276
[REDACTED]FC-SPLT - 1
24-NOV-03FOOD CONSUMPTION
MALES

RCC STUDY NUMBER 851276

FC-SPLT - 2
24-NOV-03

FOOD CONSUMPTION FEMALES

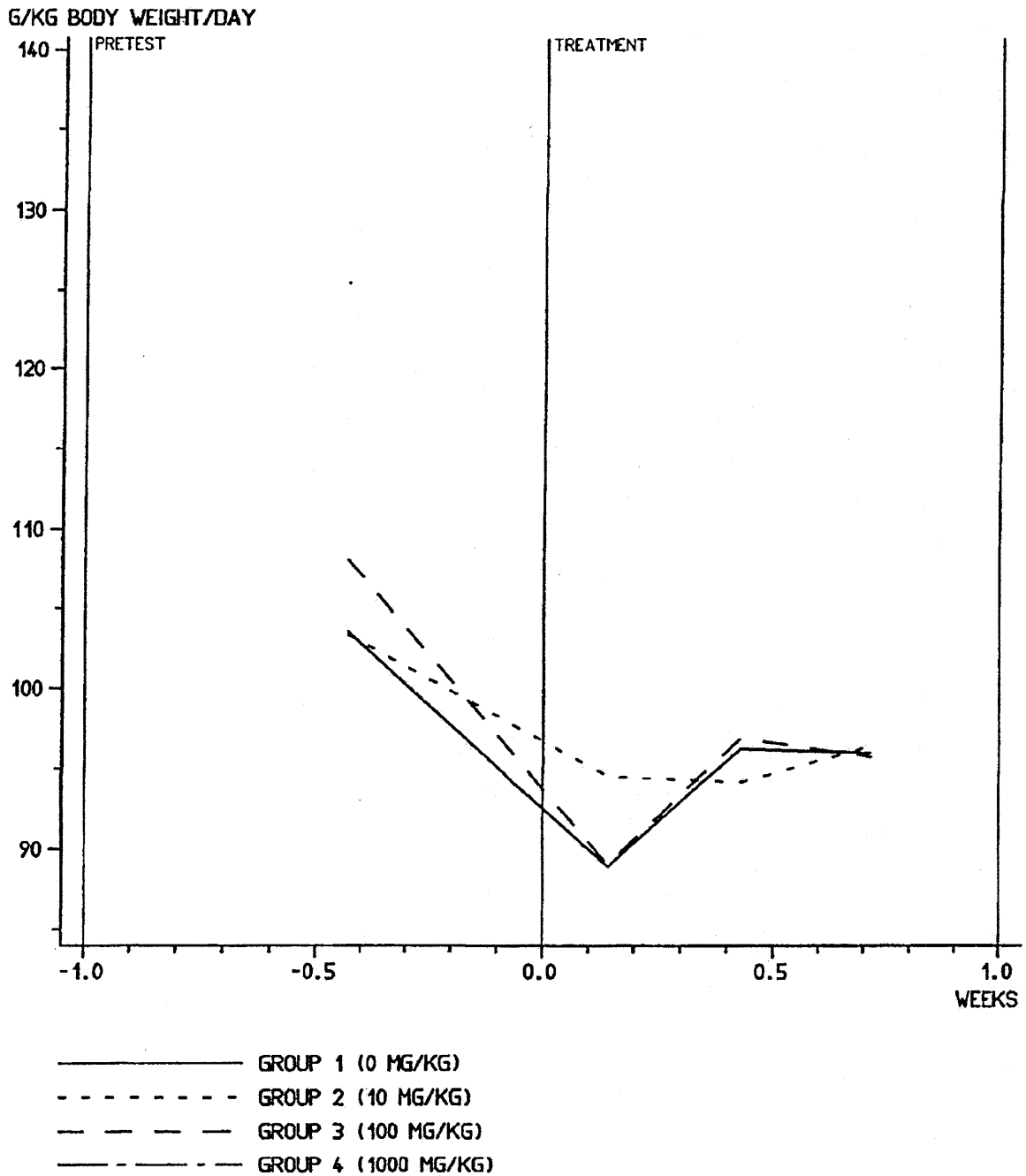


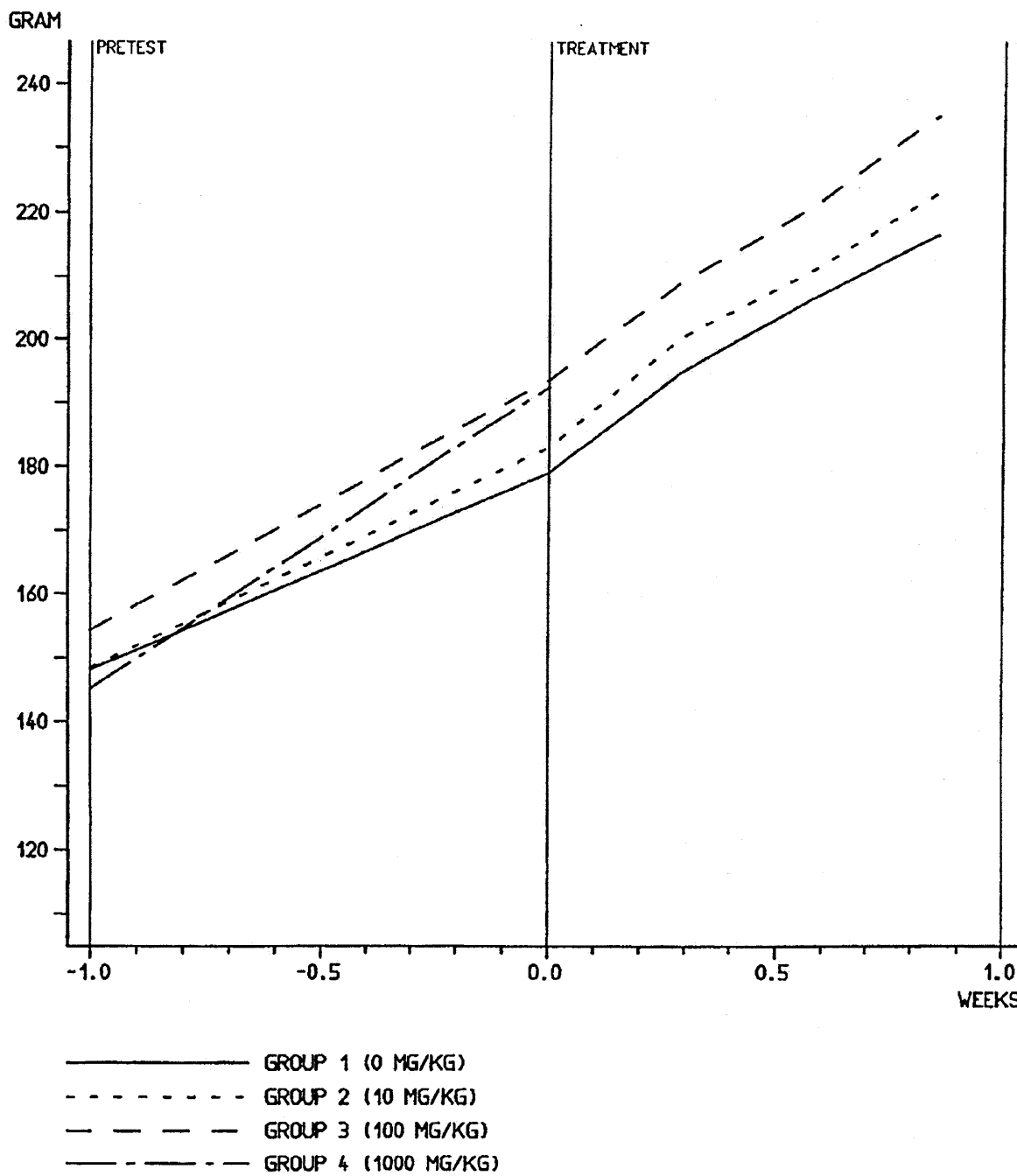
RCC STUDY NUMBER 851276
[REDACTED]RFC-SPLT - 1
24-NOV-03RELATIVE FOOD CONSUMPTION
MALES

RCC STUDY NUMBER 851276

RFC-SPLT - 2
24-NOV-03

RELATIVE FOOD CONSUMPTION FEMALES

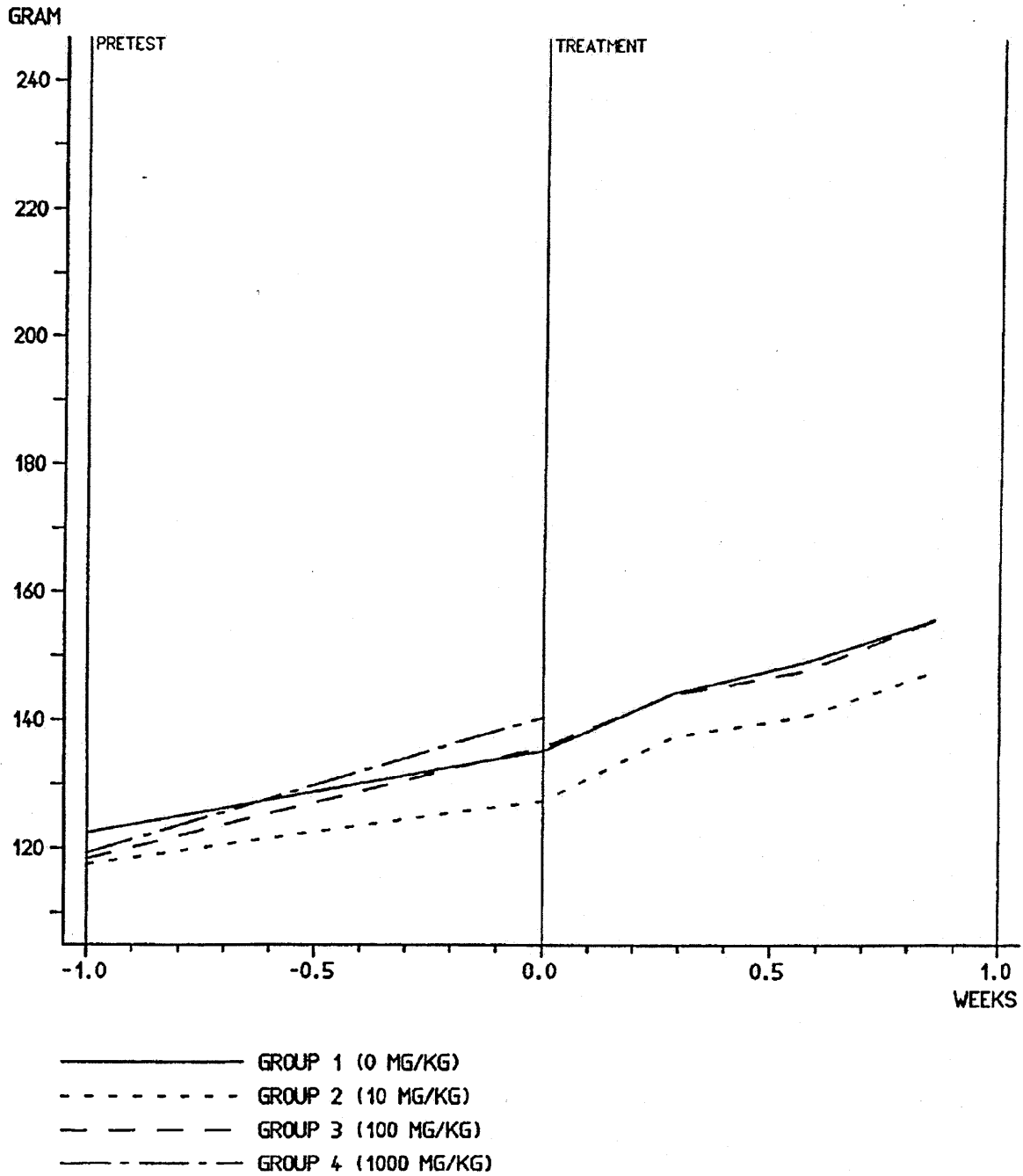


RCC STUDY NUMBER 851276
[REDACTED]BW-SPLT - 1
24-NOV-03BODY WEIGHTS
MALES

RCC STUDY NUMBER 851276

BW-SPLT - 2
24-NOV-03

BODY WEIGHTS FEMALES



RCC STUDY NUMBER 851276
[REDACTED]

SUMMARY TABLES

RCC STUDY NUMBER 851276
[REDACTED]FC-SUM - 1
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
MALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	16.6	15.4	18.9	19.7
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]FC-SUM - 2
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
MALES

TREATMENT			GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-3	MEAN		17.9	18.2	19.8	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
DAYS 3-5	MEAN		19.8	19.8	22.0	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
DAYS 5-7	MEAN		20.6	21.0	24.3	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
MEAN OF MEANS OVER TREATMENT			19.5	19.6	22.0	---

* / ** , Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]FC-SUM - 3
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
FEMALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	12.7	12.2	12.8	15.0
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]FC-SUM - 4
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
FEMALES

TREATMENT			GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS	1-3	MEAN	12.8	13.0	12.8	---
WEEK	1	ST.DEV.	---	---	---	---
		N (CAGE)	1	1	1	0
DAYS	3-5	MEAN	14.4	13.3	14.3	---
WEEK	1	ST.DEV.	---	---	---	---
		N (CAGE)	1	1	1	0
DAYS	5-7	MEAN	15.0	14.2	14.9	---
WEEK	1	ST.DEV.	---	---	---	---
		N (CAGE)	1	1	1	0
MEAN OF MEANS OVER TREATMENT			14.0	13.5	14.0	---

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]RFC-SUM - 1
24-NOV-03RELATIVE FOOD CONSUMPTION SUMMARY
(G/KG BODY WEIGHT/DAY)
MALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	112.0	103.7	122.3	136.0
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]RFC-SUM - 2
24-NOV-03RELATIVE FOOD CONSUMPTION SUMMARY
(G/KG BODY WEIGHT/DAY)
MALES

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-3	MEAN	92.0	90.7	94.5	---
WEEK 1	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	0
DAYS 3-5	MEAN	96.2	93.9	99.6	---
WEEK 1	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	0
DAYS 5-7	MEAN	95.1	94.2	103.3	---
WEEK 1	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	0
MEAN OF MEANS OVER TREATMENT		94.4	92.9	99.1	---

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]RFC-SUM - 3
24-NOV-03RELATIVE FOOD CONSUMPTION SUMMARY
(G/KG BODY WEIGHT/DAY)
FEMALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	103.5	103.4	108.1	125.5
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276

RFC-SUM - 4
24-NOV-03RELATIVE FOOD CONSUMPTION SUMMARY
(G/KG BODY WEIGHT/DAY)
FEMALES

TREATMENT			GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-3	MEAN		89.0	94.6	89.1	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
DAYS 3-5	MEAN		96.3	94.2	97.0	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
DAYS 5-7	MEAN		96.0	96.4	95.8	---
WEEK 1	ST.DEV.		---	---	---	---
	N (CAGE)		1	1	1	0
MEAN OF MEANS OVER TREATMENT			93.7	95.1	93.9	---

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

BCC STUDY NUMBER 851276
[REDACTED]BW-SUM - 1
24-NOV-03BODY WEIGHTS (GRAM) SUMMARY
MALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAY	1	148.2	148.4	154.3	145.1
WEEK	1	4.7	9.5	4.2	6.3
		N	2	2	2

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276

BW-SUM - 2

24-NOV-03

BODY WEIGHTS (GRAM) SUMMARY MALES

TREATMENT			GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAY 1	MEAN		179.1	183.0	193.6	192.4
WEEK 1	ST.DEV.		2.1	10.3	5.0	5.5
	N		2	2	2	2
DAY 3	MEAN		194.7	200.2	209.0	---
WEEK 1	ST.DEV.		1.6	11.1	3.6	---
	N		2	2	2	0
DAY 5	MEAN		206.3	210.8	220.6	---
WEEK 1	ST.DEV.		4.5	12.6	5.4	---
	N		2	2	2	0
DAY 7	MEAN		216.5	222.9	234.9	---
WEEK 1	ST.DEV.		6.2	14.1	4.7	---
	N		2	2	2	0

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]BW-SUM - 3
24-NOV-03BODY WEIGHTS (GRAM) SUMMARY
FEMALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG	
DAY	1	MEAN	122.4	117.5	118.4	119.3
WEEK	1	ST.DEV.	1.9	0.5	1.4	2.6
		N	2	2	2	2

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276

BW-SUM - 4
24-NOV-03BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT			GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
DAY 1	MEAN		135.3	127.5	135.9	140.5
WEEK 1	ST.DEV.		1.3	2.1	3.3	0.9
	N		2	2	2	2
DAY 3	MEAN		144.2	137.5	144.0	---
WEEK 1	ST.DEV.		2.0	4.9	1.2	---
	N		2	2	2	0
DAY 5	MEAN		149.1	140.6	147.7	---
WEEK 1	ST.DEV.		1.1	3.9	4.5	---
	N		2	2	2	0
DAY 7	MEAN		155.7	147.6	155.6	---
WEEK 1	ST.DEV.		1.6	5.5	2.3	---
	N		2	2	2	0

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

RCC STUDY NUMBER 851276
[REDACTED]OW-SUM - 1
25-NOV-03ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 7 DAYS
MALES

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
BODY W.	MEAN	217.94	223.05	231.96	---
	ST.DEV.	10.77	18.46	7.94	---
	N	2	2	2	0
HEART	MEAN	0.762	0.812	0.802	---
	ST.DEV.	0.003	0.114	0.004	---
	N	2	2	2	0
LIVER	MEAN	9.60	10.05	10.02	---
	ST.DEV.	0.60	1.48	0.47	---
	N	2	2	2	0
THYMUS	MEAN	0.52	0.59	0.64	---
	ST.DEV.	0.12	0.05	0.02	---
	N	2	2	2	0
KIDNEYS	MEAN	1.56	1.77	1.73	---
	ST.DEV.	0.30	0.30	0.04	---
	N	2	2	2	0
ADRENALS	MEAN	0.045	0.052	0.050	---
	ST.DEV.	0.007	0.004	0.001	---
	N	2	2	2	0
SPLEEN	MEAN	0.669	0.767	0.767	---
	ST.DEV.	0.071	0.040	0.092	---
	N	2	2	2	0
TESTES	MEAN	2.60	2.75	2.74	---
	ST.DEV.	0.11	0.33	0.10	---
	N	2	2	2	0

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

RCC STUDY NUMBER 851276

OW-SUM - 2
25-NOV-03ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 7 DAYS
MALES

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
BODY W. (GRAM)	MEAN	217.94	223.05	231.96	---
	ST.DEV.	10.77	18.46	7.94	---
	N	2	2	2	0
HEART (%)	MEAN	0.350	0.363	0.346	---
	ST.DEV.	0.019	0.021	0.010	---
	N	2	2	2	0
LIVER (%)	MEAN	4.40	4.50	4.32	---
	ST.DEV.	0.06	0.29	0.06	---
	N	2	2	2	0
THYMUS (%)	MEAN	0.24	0.26	0.28	---
	ST.DEV.	0.04	0.00	0.02	---
	N	2	2	2	0
KIDNEYS (%)	MEAN	0.71	0.79	0.75	---
	ST.DEV.	0.10	0.07	0.01	---
	N	2	2	2	0
ADRENALS (%)	MEAN	0.021	0.023	0.021	---
	ST.DEV.	0.002	0.000	0.001	---
	N	2	2	2	0
SPLEEN (%)	MEAN	0.307	0.344	0.330	---
	ST.DEV.	0.017	0.011	0.029	---
	N	2	2	2	0
TESTES (%)	MEAN	1.20	1.23	1.18	---
	ST.DEV.	0.11	0.05	0.00	---
	N	2	2	2	0

/: Dunnett-test based on pooled variance sig. at 5% or 1% level.

RCC STUDY NUMBER 851276
[REDACTED]OW-SUM - 3
25-NOV-03ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 7 DAYS
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
BODY W.	MEAN	151.86	148.22	149.96	---
	ST.DEV.	1.86	3.45	6.68	---
	N	2	2	2	0
HEART	MEAN	0.626	0.558	0.615	---
	ST.DEV.	0.012	0.001	0.008	---
	N	2	2	2	0
LIVER	MEAN	6.80	6.33	6.89	---
	ST.DEV.	0.59	0.66	0.35	---
	N	2	2	2	0
THYMUS	MEAN	0.55	0.46	0.46	---
	ST.DEV.	0.09	0.05	0.01	---
	N	2	2	2	0
KIDNEYS	MEAN	1.23	1.24	1.26	---
	ST.DEV.	0.09	0.01	0.03	---
	N	2	2	2	0
ADRENALS	MEAN	0.065	0.060	0.061	---
	ST.DEV.	0.006	0.004	0.007	---
	N	2	2	2	0
SPLEEN	MEAN	0.400	0.496	0.489	---
	ST.DEV.	0.015	0.031	0.135	---
	N	2	2	2	0

//*: Dunnett-test based on pooled variance sig. at 5% or 1% level.

RCC STUDY NUMBER 851276

OW-SUM - 4

25-NOV-03

**ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 7 DAYS
FEMALES**

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 100 MG/KG	GROUP 4 1000 MG/KG
BODY W. (GRAM)	MEAN	151.86	148.22	149.96	---
	ST.DEV.	1.86	3.45	6.68	---
	N	2	2	2	0
HEART (%)	MEAN	0.412	0.376	0.410	---
	ST.DEV.	0.013	0.008	0.013	---
	N	2	2	2	0
LIVER (%)	MEAN	4.48	4.27	4.61	---
	ST.DEV.	0.33	0.35	0.44	---
	N	2	2	2	0
THYMUS (%)	MEAN	0.36	0.31	0.31	---
	ST.DEV.	0.06	0.04	0.02	---
	N	2	2	2	0
KIDNEYS (%)	MEAN	0.81	0.84	0.84	---
	ST.DEV.	0.05	0.02	0.02	---
	N	2	2	2	0
ADRENALS (%)	MEAN	0.043	0.041	0.041	---
	ST.DEV.	0.003	0.004	0.003	---
	N	2	2	2	0
SPLEEN (%)	MEAN	0.264	0.335	0.329	---
	ST.DEV.	0.013	0.028	0.105	---
	N	2	2	2	0

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

RCC STUDY NUMBER 851276

INDIVIDUAL TABLES

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 1
25-NOV-03MORTALITY DATA
MALES
GROUP 1 (0 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
1	24-NOV-03			17-NOV-03	23-NOV-03
2	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 2
25-NOV-03MORTALITY DATA
MALES
GROUP 2 (10 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
3	24-NOV-03			17-NOV-03	23-NOV-03
4	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 3
25-NOV-03MORTALITY DATA
MALES
GROUP 3 (100 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
5	24-NOV-03			17-NOV-03	23-NOV-03
6	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 4
25-NOV-03MORTALITY DATA
MALES
GROUP 4 (1000 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
7			17-NOV-03	17-NOV-03	17-NOV-03
8		17-NOV-03		17-NOV-03	17-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 5
25-NOV-03MORTALITY DATA
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
9	24-NOV-03			17-NOV-03	23-NOV-03
10	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 6
25-NOV-03MORTALITY DATA
FEMALES
GROUP 2 (10 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
11	24-NOV-03			17-NOV-03	23-NOV-03
12	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]MORT-IND - 7
25-NOV-03MORTALITY DATA
FEMALES
GROUP 3 (100 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
13	24-NOV-03			17-NOV-03	23-NOV-03
14	24-NOV-03			17-NOV-03	23-NOV-03

RCC STUDY NUMBER 851276
MORT-IND - 8
25-NOV-03MORTALITY DATA
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL	SCHEDULED NECROPSY	SPONTANEOUS DEATH	KILLED IN EXTREMIS	TREATMENT FROM	TO
15		17-NOV-03		17-NOV-03	17-NOV-03
16		17-NOV-03		17-NOV-03	17-NOV-03

RCC STUDY NUMBER 851276
[REDACTED]FC-IND - 1
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

	PRETEST	TREATMENT		
DAYS	1-8	1-3	3-5	5-7
WEEKS	1/2	1	1	1
CAGE				
GROUP 1 (0 MG/KG)				
1	16.6	17.9	19.8	20.6
GROUP 2 (10 MG/KG)				
2	15.4	18.2	19.8	21.0
GROUP 3 (100 MG/KG)				
3	18.9	19.8	22.0	24.3
GROUP 4 (1000 MG/KG)				
4	19.7	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]FC-IND - 2
24-NOV-03FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

	PRETEST	TREATMENT		
DAYS	1-8	1-3	3-5	5-7
WEEKS	1/2	1	1	1
CAGE				
GROUP 1 (0 MG/KG)				
5	12.7	12.8	14.4	15.0
GROUP 2 (10 MG/KG)				
6	12.2	13.0	13.3	14.2
GROUP 3 (100 MG/KG)				
7	12.8	12.8	14.3	14.9
GROUP 4 (1000 MG/KG)				
8	15.0	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]RFC-IND - 1
24-NOV-03RELATIVE FOOD CONSUMPTION
(G/KG BODY WEIGHT/DAY)
MALES

	PRETEST	TREATMENT		
	1-8	1-3	3-5	5-7
DAYS	1-8	1-3	3-5	5-7
WEEKS	1/2	1	1	1
CAGE				
GROUP 1 (0 MG/KG)				
1	112.0	92.0	96.2	95.1
GROUP 2 (10 MG/KG)				
2	103.7	90.7	93.9	94.2
GROUP 3 (100 MG/KG)				
3	122.3	94.5	99.6	103.3
GROUP 4 (1000 MG/KG)				
4	136.0	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]RFC-IND - 2
24-NOV-03RELATIVE FOOD CONSUMPTION
(G/KG BODY WEIGHT/DAY)
FEMALES

	PRETEST	TREATMENT		
	1-8	1-3	3-5	5-7
DAYS				
WEEKS	1/2	1	1	1
CAGE				
GROUP 1 (0 MG/KG)				
5	103.5	89.0	96.3	96.0
GROUP 2 (10 MG/KG)				
6	103.4	94.6	94.2	96.4
GROUP 3 (100 MG/KG)				
7	108.1	89.1	97.0	95.8
GROUP 4 (1000 MG/KG)				
8	125.5	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]BW-IND - 1
24-NOV-03BODY WEIGHTS (GRAM)
MALES

	PRETEST	TREATMENT				
DAYS	1	1	3	5	7	
WEEKS	1	1	1	1	1	
ANIMAL						
GROUP 1 (0 MG/KG)						
1	151.5	180.6	193.6	203.1	212.1	
2	144.8	177.6	195.9	209.5	220.9	
GROUP 2 (10 MG/KG)						
3	155.2	190.3	208.1	219.7	232.9	
4	141.7	175.7	192.4	201.9	213.0	
GROUP 3 (100 MG/KG)						
5	151.3	197.1	211.6	224.4	238.3	
6	157.3	190.1	206.4	216.8	231.6	
GROUP 4 (1000 MG/KG)						
7	140.7	188.6	---	---	---	
8	149.6	196.3	---	---	---	

RCC STUDY NUMBER 851276
[REDACTED]BW-IND - 2
24-NOV-03BODY WEIGHTS (GRAM)
FEMALES

	PRETEST	TREATMENT			
DAYS	1	1	3	5	7
WEEKS	1	1	1	1	1
ANIMAL					
GROUP 1 (0 MG/KG)					
9	123.7	136.2	145.6	149.8	156.8
10	121.0	134.4	142.8	148.3	154.6
GROUP 2 (10 MG/KG)					
11	117.2	129.0	140.9	143.4	151.5
12	117.9	126.0	134.0	137.9	143.7
GROUP 3 (100 MG/KG)					
13	119.3	138.2	144.8	150.9	157.2
14	117.4	133.6	143.1	144.6	154.0
GROUP 4 (1000 MG/KG)					
15	117.4	139.8	---	---	---
16	121.1	141.1	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 1
25-NOV-03

MACROSCOPICAL FINDINGS
MALES
GROUP 1 (0 MG/KG)

ANIMAL 1

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 2

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 2
25-NOV-03

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (10 MG/KG)

ANIMAL 3

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 4

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 3
25-NOV-03

MACROSCOPICAL FINDINGS
MALES
GROUP 3 (100 MG/KG)

ANIMAL 5

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 6

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 4
25-NOV-03

MACROSCOPICAL FINDINGS
MALES
GROUP 4 (1000 MG/KG)

ANIMAL 7

(KILLED IN EXTREMIS, 17-NOV-2003)

NO FINDINGS NOTED

ANIMAL 8

(SPONTANEOUS DEATH, 17-NOV-2003)

NO FINDINGS NOTED

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 5
25-NOV-03

MACROSCOPICAL FINDINGS
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL 9

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 10

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

BCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 6
25-NOV-03

MACROSCOPICAL FINDINGS
FEMALES
GROUP 2 (10 MG/KG)

ANIMAL 11

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 12

(SCHEDULED NECROPSY, 24-NOV-2003)

KIDNEYS..... BOTH SIDES: PELVIC DILATION.

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 7
25-NOV-03

MACROSCOPICAL FINDINGS
FEMALES
GROUP 3 (100 MG/KG)

ANIMAL 13

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

ANIMAL 14

(SCHEDULED NECROPSY, 24-NOV-2003)

NO FINDINGS NOTED

RCC STUDY NUMBER 851276
[REDACTED]

MAC-IND - 8
25-NOV-03

MACROSCOPICAL FINDINGS
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL 15

(SPONTANEOUS DEATH, 17-NOV-2003)

NO FINDINGS NOTED

ANIMAL 16

(SPONTANEOUS DEATH, 17-NOV-2003)

THYMUS..... FOCUS/FOCI, ISOLATED, D=1 MM, DARK RED.

RCC STUDY NUMBER 851276

OW-IND - 1
25-NOV-03ORGAN WEIGHTS (GRAM)
AFTER 7 DAYS
MALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
1	210.32	0.764	9.18	0.43	1.35	0.041	0.620	2.67
2	225.55	0.759	10.02	0.61	1.77	0.050	0.719	2.52

GROUP 2 (10 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
3	236.10	0.893	11.10	0.63	1.98	0.055	0.795	2.98
4	210.00	0.731	9.01	0.55	1.56	0.049	0.739	2.51

GROUP 3 (100 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
5	237.57	0.804	10.35	0.63	1.76	0.049	0.832	2.81
6	226.34	0.799	9.69	0.66	1.71	0.050	0.701	2.67

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
7	---	---	---	---	---	---	---	---
8	---	---	---	---	---	---	---	---

RCC STUDY NUMBER 851276

OW-IND - 2
25-NOV-03ORGAN/BODY WEIGHT RATIOS (%)
AFTER 7 DAYS
MALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
1	210.32	0.363	4.36	0.21	0.64	0.019	0.295	1.27
2	225.55	0.337	4.44	0.27	0.78	0.022	0.319	1.12

GROUP 2 (10 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
3	236.10	0.378	4.70	0.27	0.84	0.023	0.337	1.26
4	210.00	0.348	4.29	0.26	0.74	0.023	0.352	1.20

GROUP 3 (100 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
5	237.57	0.339	4.36	0.26	0.74	0.021	0.350	1.18
6	226.34	0.353	4.28	0.29	0.75	0.022	0.310	1.18

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
7	---	---	---	---	---	---	---	---
8	---	---	---	---	---	---	---	---

RCC STUDY NUMBER 851276

OW-IND - 3

25-NOV-03

ORGAN WEIGHTS (GRAM)
AFTER 7 DAYS
FEMALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
9	150.54	0.635	6.38	0.48	1.17	0.061	0.411
10	153.17	0.618	7.22	0.62	1.29	0.069	0.390

GROUP 2 (10 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
11	150.66	0.558	6.80	0.42	1.24	0.057	0.474
12	145.78	0.557	5.86	0.49	1.24	0.063	0.518

GROUP 3 (100 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
13	145.23	0.609	7.14	0.47	1.24	0.056	0.585
14	154.68	0.620	6.65	0.46	1.28	0.067	0.394

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W.	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
15	---	---	---	---	---	---	---
16	---	---	---	---	---	---	---

RCC STUDY NUMBER 851276
[REDACTED]OW-IND - 4
25-NOV-03ORGAN/BODY WEIGHT RATIOS (%)
AFTER 7 DAYS
FEMALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
9	150.54	0.422	4.24	0.32	0.77	0.040	0.273
10	153.17	0.403	4.71	0.40	0.84	0.045	0.254

GROUP 2 (10 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
11	150.66	0.371	4.51	0.28	0.82	0.038	0.315
12	145.78	0.382	4.02	0.34	0.85	0.043	0.355

GROUP 3 (100 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
13	145.23	0.419	4.92	0.32	0.85	0.039	0.403
14	154.68	0.401	4.30	0.30	0.83	0.043	0.255

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
15	---	---	---	---	---	---	---
16	---	---	---	---	---	---	---